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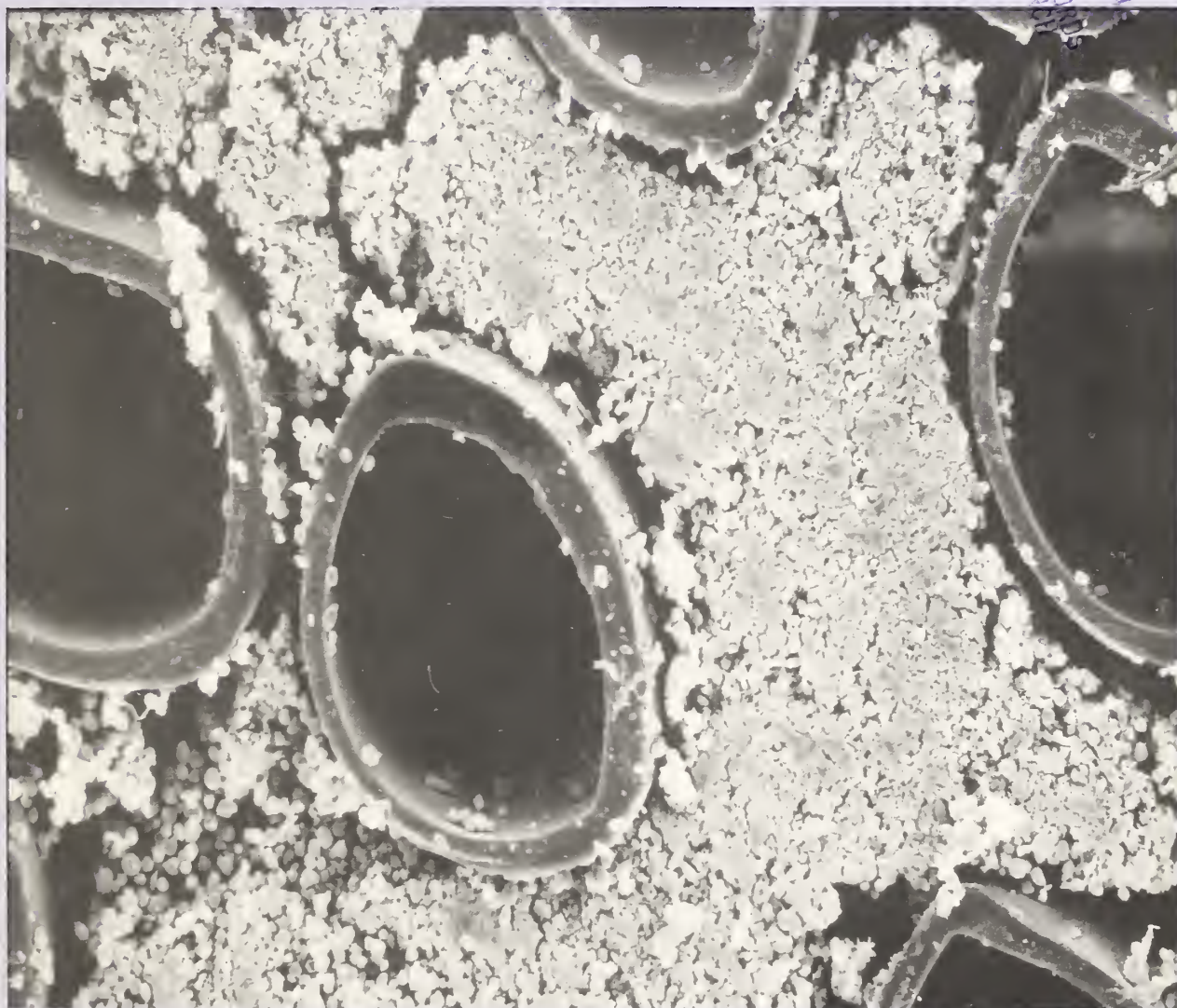


Information Resources for Adjuvants and Antibody Production

Comparisons and Alternative Technologies

1990 - 1997

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Information Resources for Adjuvants and Antibody Production: Comparisons and Alternative Technologies 1990 - 1997

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10301 Baltimore Avenue
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Telephone: 301-504-6212
Fax: 301-504-7125
E-mail: awic@nal.usda.gov
Jean Larson, Coordinator

Editors:

Cynthia Petrie Smith, M.S.
D'Anna Jensen, B.S.
Tim Allen, M.S.
Michael Kreger, M.S.

Articles contributed by:

W.C. Hanly, University of Illinois, USA
B.Taylor Bennett, University of Illinois, USA
James E. Artwohl, University of Illinois, USA
Uwe Marx, University of Leipzig, Germany
M. Jim Embleton, Christie Hospital NHS Trust, UK
René Fischer, ETH-Zentrum, Switzerland
Franz P. Gruber, FFVFF, Switzerland
Ulrika Hansson, Swedish Fund for Research without Animal Experiments, Sweden
Joachim Heuer, ZEBET, Germany
Wim A. de Leeuw, Ministry of Public Health, Welfare and Sport, The Netherlands
Ton Logtenberg,, University Hospital Utrecht, The Netherlands
Wolfram Merz, INTEGRA Biosciences GmbH, Germany
Daniel Portetelle, Faculty of Agronomy, Gembloux, Belgium
John-Louis Romette, Université de la Méditerranée, France
Donald W. Straughan, FRAME, UK

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Cover photo provided by Celco, Incorporated -- a cross-section of an artificial capillary (hollow fiber) cartridge filled with lymphocytes as seen under a scanning electron microscope. Cells are growing at a density of 10^8 /ml. This is the type of growth seen with other lymphoid cell types (i.e. hybridoma).

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Introduction

Information Resources for Adjuvants and Antibody Production: Comparisons and Alternative Technologies was developed by the Animal Welfare Information Center (AWIC) of the U.S. Department of Agriculture (USDA), National Agricultural Library (NAL), in an effort to provide current information to assist Institutional Animal Care and Use Committees (IACUCS) in evaluating protocols involving the use of animals in antibody production. Concern for the welfare of animals used in antibody production has been focused in two major areas; the choice of adjuvants used to illicit an immune response; and the use of ascites production.

Adjuvants are substances that enhance the body's immune response to an antigen. Careful selection and use of adjuvants is necessary to prevent adverse side-effects such as inflammation and lesioning. Ascites production is the process whereby hybridoma cells are injected into the peritoneal cavity of primarily rats or mice. Monoclonal antibodies are then harvested from ascitic fluid of injected animals. Frequent monitoring of test animals is necessary to minimize pain and distress.

In recent years, an increasing number of papers have been published comparing adjuvants, in vitro antibody production methods, and other technologies. In the following document, writings by distinguished researchers comparing traditional and alternative methods introduce the adjuvant and antibody sections. Each introduction is followed by an extensive bibliography compiled from scientific journals, proceedings, and newsletters. Emphasis was placed on citing comparative and/or alternative methods research. A company/institute listing of selected suppliers of alternative adjuvants, antibodies produced using new technologies, and novel cell culture products is listed as well. Other sections include a listing of educational web site resources, organizations, and a subject index.

The staff of the Animal Welfare Information Center hope that you find this publication to be a useful addition to your laboratory animal resources and welcome any comments for future editions.

How To Use This Document

The following publication is divided into 7 sections: articles and bibliographies, a listing of books and proceedings, a selected list of company and institute resources, educational web site resources, organizations, subject index, and document delivery information for U.S. and foreign patrons.

Articles and Bibliographies

Each section on adjuvants and antibody production is preceded by an article written by a recognized authority in the field. Immediately following each article is a comprehensive bibliography containing citations that are arranged alphabetically according to the last name of the primary author. The reference section of each article may or may not overlap with citations in the bibliographies. Each bibliographic entry contains descriptors and the NAL Call Number if the particular source is available at the National Agricultural Library (NAL). International Standard Serial Numbers (ISSN) and International Standard Book Numbers (ISBN) have also been included to assist patrons in locating publications from their own libraries.

Books and Proceedings

This section consists of a listing of books and proceedings that are of interest to researchers in the antibody production field. Many of the publications are not easily found in bibliographic databases and are excellent resources that patrons may not be aware of. Each entry includes author, title, publisher name, ISBN, and NAL Call Number.

Selected List of Company and Institute Resources Providing New Technologies

This section was developed to assist patrons in contacting companies and research institutes that produce novel adjuvants and/or antibodies using alternative methods (i.e. in vitro, phage display). Although many companies and institutes listed in the U.S. still provide monoclonal antibodies via ascites production in mice, some companies are beginning to offer in vitro or novel services as well. All contact information is current as of June, 1997. Due to the dynamic state of information on the internet, e-mail and URL addresses listed may or may not be functional in the future. Please note that "800" telephone numbers for U.S. companies may not be reached by all countries. Inclusion of a company or institute does not constitute an official endorsement or approval by the U.S.D.A., Agricultural Research Service.

Educational Web Site Resources

This section cites: general antibody resources, articles, references, faculty home pages, course information, and conference information available on the internet. It is hoped that this section will aid researchers, Institutional Animal Care and Use Committee (IACUC) members and others, in sifting through the myriad of sites on the internet more efficiently.

Organizations

A number of organizations world wide have shown an interest in antibody production related issues. In this section, organizations are listed by world regions. Information on how to contact each organization via a variety of electronic means and/or postal address is provided. The type of organization, resources or services offered, requester priority, and fees (if any), are also listed.

Subject Index

The index for this publication was generated primarily from the descriptors that accompany each entry. In some instances, index words may have been taken from the title. The number associated with each index term corresponds to the *page number* on which the index term can be found.

Document Delivery Information

The information contained here provides directions on how to obtain copies of articles mentioned in the bibliography. There are separate directions for U.S. patrons and those readers outside the United States. **All patrons are encouraged to use their local resources before contacting the National Agricultural Library.** While the National Agricultural Library provides a variety of services to patrons around the world, videocassettes are not available for loan outside the United States and Canada.

Overview of Adjuvants

W. C. Hanly, Ph.D., Department of Microbiology and Immunology,
B. Taylor Bennett, D.V.M., Ph.D., and James E. Artwohl, D.V.M.,
Biologic Resources Laboratory,
College of Medicine, University of Illinois
Chicago, Illinois

The following was adapted from a series of short articles published in the August and September 1994 issues of the *BRL Bulletin*. These articles were intended to explain briefly the function of adjuvants; guidelines for use of adjuvants, particularly Freund's adjuvants; and to introduce alternative adjuvants. For a more in depth discussion of adjuvants and antibody production patrons are referred to the *ILAR Journal*, volume 37, number 3, 1995.

Need for adjuvants

The production of antibodies [Ab] in laboratory animals is a tool used in many fields of biomedical research. Antibodies are routinely made to proteins, carbohydrates, complex lipids, and nucleic acids isolated from natural sources. In addition, modern biochemical, biosynthetic, and recombinant DNA techniques have created increasingly pure antigens [Ag]. Many of these newer antigens are small or generally weak immunogens. The small polypeptides (< 10 kDa) and nonprotein antigens usually need to be conjugated to a large immunogenic carrier protein to become good immunogens. These as well as most other protein antigens (especially when administered in small quantity) need to be administered with an adjuvant to assure a high quality/high quantity, memory-enhanced antibody response by the laboratory animal. In the past few years a number of new adjuvants have become available for use in laboratory animals, although Freund's adjuvants continue to be the most commonly used despite their potential hazards.

Functions of adjuvants

In general, μ g to mg quantities of a protein Ag are needed to elicit an antibody response in a laboratory animal. This range, which may differ from Ag to Ag and from species to species, is called the "window of immunogenicity". Too much or too little Ag may induce tolerance rather than an active response for the given Ag. Compared to injection of Ag alone, injection of antigen plus an adjuvant generally permits use of a much smaller quantity of the Ag and greatly enhances the Ab titer (Kaeberle, 1986). Within the window of immunogenicity, with or without adjuvant, larger Ag doses generally result in greater Ab responses up to a point at which suppressive activities become exaggerated. However, production of the highest titer is not always the best goal, for a moderate titer of high affinity Ab may be preferable to a high titer of low affinity. High affinity Ab generally results from immunization with smaller quantities of Ag than needed for production of the highest titer.

For an animal to sustain an Ab response, a continual or intermittent supply of Ag is needed. One way an adjuvant may aid the immune response is by forming a depot of Ag at the injection site resulting in the sustained release of small quantities of Ag over a long period of time. This approach gives sustained stimulation while minimizing suppressive effects. Even with an adjuvant that forms a depot of Ag, at some point in time the quantity of Ag is diminished and the Ab titer declines. At this time a second injection of Ag (a booster dose) may be given. When an animal that has responded maximally is given a booster dose of Ag too soon, suppression rather than enhancement of the immune response may ensue. Ideally, one follows the serum Ab titer in a hyperimmunized animal and gives a booster injection of Ag only after the Ab titer has begun to decline. However, when an animal has responded less than maximally (which is the more usual situation for small doses of Ag), a booster dose of Ag given at 3 to 6 weeks after the first Ag dose will usually increase the serum Ab titer (Cooper et al., 1991; Siskind et al., 1968; Herbert, 1978; Hu and Kitagawa, 1990). Booster doses of Ag are typically equal to or less than ($\sim 1/2$) the priming dose. The advantage of using a smaller Ag dose is that only the higher affinity clones of B cells are stimulated, thus improving the quality of the Ab produced.

A second way an adjuvant can work is to serve as a vehicle to help deliver the Ag to the spleen and/or lymph nodes where Ag is trapped by the follicular dendritic cells and where most of the necessary cell to cell interactions take place to generate plasma cells (the Ab-secreting cells). For example, microdroplets of oil containing Ag, such as those formed in an oil-in-water adjuvant emulsion, are readily ingested by macrophage and taken to draining lymph nodes or spleen. Additionally, emulsions aid tissue dendritic cells in their capture of Ag. Ag-loaded tissue dendritic cells rapidly emigrate via lymphatics to draining lymph nodes. Substances that activate complement enhance trapping of Ag by the follicular dendritic cells through their surface complement receptors. It is, in fact, the retention of Ag in the follicles of the spleen and/or lymph nodes that is essential for Ab production and for the maintenance of memory. The terminally differentiated plasma cells survive only a few days to a few weeks, and thus must be replaced by reactivated memory cells. Memory cell activation takes place only in the Ag-loaded follicles.

A third way an adjuvant can work is to activate the various cells involved in the immune response, either directly or indirectly. Surfactants, components of all emulsion adjuvants, may serve this function as well as helping to stabilize oil-water emulsions. Also, many bacteria contain substances that activate cells of the immune system, particularly the macrophage. The activated macrophage in turn helps activate T and B cells. Thus some adjuvants contain bacteria, bacterial products, or derivatives of bacterial products. Although the activation of macrophages indeed aids in the antibody response, excessive activation of macrophages also causes excessive inflammation, so that bacterial components cannot be used in excess. In recent years, a number of bacterial products have been modified in ways that maximize their desirable activation potential and minimize their inflammatory potential with the goal of finding ideal adjuvant components. For example, some of the new generation adjuvants incorporate a chemical variant of endotoxin called monophosphoryl lipid A [MPL] or a modified muramyl dipeptide [thr-MDP] or other "detoxified" cell wall constituents of bacteria (Rudbach et al., 1988).

Advantages and disadvantages of Freund's adjuvants

Freund's Complete Adjuvant (FCA), a mixture of a non-metabolizable oil (mineral oil), a surfactant (Arlacel A), and mycobacteria (*M. tuberculosis* or *M. butyricum*) has been used for many years to enhance immunologic responses to antigens, and even today is considered to be one of the most effective adjuvants. It is prepared as a water-in-oil emulsion by combining one volume FCA with one volume aqueous antigen solution. In the emulsion, Ag is distributed over a large surface area thereby increasing the potential for interaction with relevant cells. Antibody production is enhanced by FCA primarily because of: a) the depot effect and b) nonspecific immunopotential of macrophages by surfactant and the mycobacteria.

The preparation of an appropriate and stable emulsion is critical for the proper functioning of the adjuvant, and methods to prepare the emulsion and check its stability have been described (Herbert, 1978; Moncada et al., 1993). Some important points are emphasized here. A source bottle of FCA needs to be mixed very thoroughly before an aliquot is withdrawn in order to assure even distribution of the mycobacteria. Then the aqueous phase, in stepped portions, is introduced into the oil, rather than vice-versa, assuring that the oil becomes the continuous phase. Mixing follows addition of each portion of aqueous phase and can be accomplished with two glass syringes connected with a double hubbed needle or a 3-way stopcock (the latter being advantageous for stepped addition of the aqueous phase by a third syringe). The goal is to achieve water droplets $< 1 \mu\text{m}$ diameter dispersed in the continuous oil phase without introduction of air. A drop of a stable emulsion will remain cohesive when floated on cold water.

Although FCA is a very effective adjuvant for production of antibodies, there are problems and hazards associated with its use (Broderick, 1989; Kleinman et al., 1993; Claassen et al., 1992; Steiner JW et al, 1960; Stills and Bailey, 1989; Stills 1994). At the site of injection, FCA causes a chronic inflammatory response that may be severe and painful for the animal depending on the site as well as the quantity and quality of adjuvant injected. The inflammatory response may result in formation of chronic granulomas, sterile abscesses, and/or ulcerating tissue necrosis. Adjuvant-induced lesions may appear to be metastatic when excessive amounts of the emulsion are injected in a single site. Emulsion injected subcutaneously on the dorsal region of some species (rabbit, in particular) may migrate by fistulous tracts to the ventral region of the animal. Emulsion injected intramuscularly may spread along fascial planes to distant muscular sites or may travel to lung, liver or other organs, apparently by a hematogenous route. Monitoring the site of injection as an index of health of the animal may be inadequate when excessive quantities of emulsion are injected.

Measures to limit the severity of the inflammatory reaction include: a) choosing or making preparations of FCA with a lower mycobacterial concentration, i.e., 0.05 to 0.1 mg/ml, rather than 1 mg/ml; b) adding a concentrated antigen solution to the adjuvant to obtain an antigen-rich emulsion, thereby reducing the quantity of emulsion injected; c) using multiple injection sites with limitation of volume injected at any one site; d) separation of injection sites to avoid fusion of inflammatory lesions; and e) maintaining sterility of the Ag solution.

FCA is also a potential hazard for laboratory personnel. Accidental self-inoculation can result in tuberculin sensitization followed by chronic local inflammation which responds poorly to antibiotic treatment (Chapel and August, 1976). Accidental splashing of FCA in the eye, a risk for unguarded eyes during preparation of the emulsion or during injection of the animal, can result in

severe ocular irritation and even blindness. For individuals already sensitized to mycobacterial antigens (i.e., those having a positive tuberculin skin test), an accidental inoculation can cause a severe necrotizing lesion that persists for months or that may require surgical excision of the injection site for ultimate resolution. Systemic effects persisting for weeks or months such as fever and neurological and arthritic symptoms have also been described.

Freund's Incomplete Adjuvant (FIA) has the same oil/surfactant mixture as FCA but does not contain any mycobacteria. It is frequently used to boost animals that received a primary antigen injection in FCA, but it can be used as the adjuvant for the primary injection as well. It has adjuvant properties that favor humoral immunity without cell-mediated immunity, but is generally considered to be less potent than FCA (although exceptions exist). FIA is capable of causing abscesses and granuloma formation, but such reactions are generally less severe than those that accompany the use of FCA.

Prior to using Freund's adjuvants, particularly FCA, investigators should consider the use of an alternative adjuvant system. Several new generation adjuvants are commercially available, and some others may be constructed in an investigator's laboratory.

Alternative adjuvants

Montanide ISA Adjuvants [Seppic, Paris, France] are a group of oil/surfactant based adjuvants in which different surfactants are combined with either a non-metabolizable mineral oil, a metabolizable oil, or a mixture of the two. They are prepared for use as an emulsion with aqueous Ag solution. The surfactant for Montanide ISA 50 [ISA = Incomplete Seppic Adjuvant] is mannide oleate, a major component of the surfactant in Freund's adjuvants. The surfactants of the Montanide group undergo strict quality control to guard against contamination by any substances that could cause excessive inflammation, as has been found for some lots of Arlacel A used in Freund's adjuvant. The various Montanide ISA group of adjuvants are used as water-in-oil emulsions, oil-in-water emulsions, or water-in-oil-in-water emulsions. The different adjuvants accommodate different aqueous phase/oil phase ratios, because of the variety of surfactant and oil combinations. The performance of these adjuvants is said to be similar to Incomplete Freund's Adjuvant [IFA] for antibody production; however the inflammatory response is usually less.

Ribi's Adjuvants (Ribi ImmunoChem Research, Inc., Hamilton, MT) are supplied by the manufacturer as mixtures of oil, detergent, and immunostimulator(s); the investigator need only add aqueous Ag and mix by vortexing to form a stable oil-in-water emulsion. To minimize the inflammatory response, the adjuvant utilizes a metabolizable oil (squalene) and contains modified bacterial products designed to provide immunopotential without excessive inflammation (Rudbach et al., 1988). One mycobacterial component, trehalose dimycolate [TDM], serves as a surfactant and an immunostimulator as well as an adherence factor in binding protein Ag to the oil droplets. Also, because significantly less oil is needed for a stable oil-in-water emulsion than for a stable water-in-oil emulsion, a Ribi's adjuvant presumably causes less tissue damage than water-in-oil adjuvants, since less oil is administered. However, the depot effect is not as great as with water-in-oil emulsions and thus booster injections are needed more frequently. Ribi's adjuvants provide a choice of different detoxified bacterial products as immunostimulators for

different species, and thus the manufacturer refers to the products as Ribi's Adjuvant System [RAS]. A Ribi's adjuvant may not be applicable to all antigens or all species, but may in some cases be superior to FCA. Variable results have been reported, but experience with this adjuvant is not as extensive as with FCA. In general, a Ribi's adjuvant emulsion (or other oil-in-water emulsion) is better for protein antigens that have some hydrophobic aspects or are amphipathic than for very hydrophilic proteins. This is because the adjuvant's effectiveness is dependent on adsorption of the protein antigen to the oil droplets of the oil-in-water emulsion. This mode of antigen presentation to B cells should bias the antibody response to epitopes of the native protein rather than to epitopes of the denatured protein. You should consider whether antibodies to native or denatured protein antigen are desired when choosing an adjuvant.

Hunter's TiterMax (CytRx Corp., Norcross, GA) is an oil/surfactant-based adjuvant prepared as a water-in-oil emulsion in a manner similar to that used for Freund's adjuvants. However, it uses a metabolizable oil (squalene) and a nonionic surfactant that has good protein antigen-binding capacity as well as adjuvant activity. The adjuvant activity may relate, in part, to the surfactant's ability to activate complement and bind complement components, as this helps target the Ag to follicular dendritic cells in the spleen and lymph nodes. The surfactant used in the commercially available adjuvant is one of a number of synthetic nonionic block copolymers of polyoxyethylene and polyoxypropylene developed by Hunter (Hunter et al., 1991). It was found to be superior for most, but not all proteins. Although experience with TiterMax is limited, some reports show it to be superior or equal to Freund's adjuvants with some protein antigens, particularly in rabbits and mice (Bennett et al, 1992). It may not be as successful in rats. When compared to FCA, TiterMax can be used in smaller quantities for initial injections, which minimizes the inflammatory reaction at the injection site. The inflammatory reaction that does develop is said to be due primarily to an Arthus type reaction. The utilization of copolymer-coated microparticles to stabilize the emulsion permits formation of stable emulsions with less than 20% oil, a big factor in minimizing total adjuvant injected. Also, booster injections may be needed less frequently than with Freund's adjuvant, making TiterMax after the initial investment, a cost effective adjuvant for antibody production.

Aluminum Salt Adjuvants are used with protein antigens in two ways: a) as alum-precipitated vaccines and b) as alum-adsorbed vaccines (Harlow and Lane, 1988; Nicklas, 1992). Investigator generated or commercially available $\text{Al}(\text{OH})_3$ [Alhydrogel - Superfos of Denmark/Accurate Chemical and Scientific Co., Westbury, NY] can be used to adsorb proteins in a ratio of 50 - 200 μg protein/mg aluminum hydroxide. Adsorption of protein is dependent on the pI (Isoelectric pH) of the protein and the pH of the medium. A protein with a lower pI adsorbs to the positively charged aluminum ion more strongly than a protein with a higher pI. Aluminum salts are generally weaker adjuvants than emulsion adjuvants; however, because of their generally mild inflammatory reactions, safety, and efficacy for generating memory, they are the primary adjuvants utilized in humans. When used in larger quantity in laboratory animals, the inflammatory reactions that may occur at the site of injection will generally resolve within a few weeks although chronic granulomas may occasionally form. The mineral adjuvants work by establishing a depot of Ag which is released slowly over a period of 2-3 weeks, nonspecific activation of macrophages and complement activation. The effectiveness of aluminum salt adjuvants has been increased in

experimental studies by the addition of gamma-inulin (Cooper et al., 1991), detergents, or *Bordetella pertussis*, but the inflammatory reaction is generally increased as well. Due to the short-term depot effect, the alum booster injections may be needed more frequently than with water-in-oil emulsions.

Nitrocellulose-Adsorbed Protein can be used for immunization without desorption of the protein in vitro, as desorption of protein from nitrocellulose [NC] paper will occur in vivo giving a desirable slow release of Ag over a period of 2 weeks to 2 months (Nilsson and Larsson, 1992). The nitrocellulose itself is essentially inert, causing little if any inflammatory response and no anti-NC antibody response. Either intrasplenic or subcutaneous deposition of the nitrocellulose paper with ng to μ g quantities of Ag has been successfully used for antibody production. On the order of 100 μ g of protein can bind to 1 cm² of NC, so that only small quantities of NC need be introduced into the animal. The antibody response is not as vigorous as with FCA/antigen emulsions and may require 1 or more booster immunizations to be detected, but this method is particularly advantageous for situations in which only small quantities of pure Ag can be obtained as in a band from an electroblot. Antibodies have even been raised to NC-adsorbed protein administered after the protein had been stained with Coomassie Blue. However, you should be aware that antibodies raised to reduced or otherwise denatured protein may not react well with the native protein. On the other hand, such antibodies may be desirable for use on electroblots (Western blots).

Encapsulated Antigens have been prepared in several ways permitting sustained slow release of Ag and, in some cases, release of immunostimulators as well. Examples include liposome-entrapped Ag, nondegradable ethylene-vinyl acetate copolymer [EVAc]-entrapped Ag (Niemi et al., 1985), and degradable polymer-entrapped Ag. Among the biodegradable, biocompatible polymers used for encapsulation, poly(DL-lactide-co-glycolide) demonstrates very favorable characteristics for use in bulk-prepared vaccines, in that construction and administration of different size microspheres in a single dose may accomplish timed release of Ag in a way that mimics primary and booster injections (Eldridge et al., 1991). However, the preparation is rather complex for use with the occasional antigens prepared for injection by individual investigators, particularly when the antigen is available in very limited quantity. The complexity of preparation is a drawback for essentially all of the encapsulated Ag preparations, but in special situations the potential of an encapsulated Ag to generate a significant immune response may make its preparation worthwhile.

Gerbu Adjuvant [Gerbu Biotechnik GmbH, Gaiberg, Germany/C-C Biotech, Poway, CA] is a new aqueous phase adjuvant that does not have a depot effect. It utilizes immunostimulators in combination with zinc proline. Although it requires frequent boosting to achieve a high-titered response, the inflammatory effect at the site of injection is minimal. Preliminary experiments reported by a member of the company indicate that the adjuvant can be safely used for footpad injection of rabbits.

Preparation of Ag solutions for injection

When the aqueous Ag is prepared for vaccine use, it is important that it be prepared in a manner to prevent or eliminate contamination, particularly extraneous bacteria or bacterial products that may cause sepsis or extensive inflammatory reaction. Most protein antigens can be filtered through a microporous filter (0.22 μm pore size) of a type that has minimal adsorption of protein to achieve sterility of the preparation just before its addition to adjuvant. It is also important for you to take measures at all stages of Ag preparation to minimize contamination of the preparation by bacterial endotoxin which is inflammatory and pyrogenic and may not be removed by filtration.

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Descriptors: *Quillaja saponaria*, South American soap tree, toxic, rabies vaccine, variations in available preparation.
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Descriptors: HIV vaccine, alum, cell-wall derived adjuvants, cytokines, carriers and vehicles, T-cell response, mucosal immunity.
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Descriptors: rabbit, alpha 2-macroglobulin, free hen egg lysozyme (HEL), T hybridoma clones, in vitro, rabbits, subcutaneous immunization, nitrocellulose paper strips.

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Descriptors: antigen hydrophobicity, cytokine production, vehicles, pain.
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Descriptors: water-in-oil emulsions, side effects, comparisons with other adjuvants.
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Descriptors: Quil-A, immune stimulating complex (iscom), production process, *Herpesviridae*, Hepatitis B virus, rubella, rabies.
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NAL call number: 410.9 P94
Descriptors: polyclonal antibody, golf ball, titer quantity, compare with Freund's or acylamide.
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Descriptors: polyclonal antibodies, subcutaneous implant, antigen purity, monoclonal, inflammation, discomfort.
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Descriptors: trout, Freund's complete adjuvant, muramyl dipeptide, adjuvant combinations, titer, aquaculture.

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Descriptors: polysaccharide-diphtheria toxoid conjugate, detoxified, lipopolysaccharide, female, ICR mice, saponin adjuvant, tree bark.
- Davidson, E., and E. Prentice (1994). **TiterMax an adjuvant update.** Vaxcel, Inc.: Norcross, GA, 1 videocassette (18 min.), 1/2 in.
NAL call number: Videocassette no.2038
Descriptors: alternative adjuvant, less toxic, emulsification procedures, step-by-step visual preparation.
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NAL call number: QR180 J6
Descriptors: IgG, IgM, rabbit, Ribi, Freund's, inflammation, discomfort.
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Descriptors: mice, cytokines, toxicity, complete Freund's adjuvant, leukopenia, neutropenia, interleukin-6, lipopolysaccharides, tumor necrosis factor.
- Degling, L. and P. Stjarnkvist P. (1995). **Biodegradable microspheres XVIII: The adjuvant effect of polyacryl starch microparticles with conjugated human serum albumin.** *Vaccine* 13(7):629-636, ISSN:0264-410X.
NAL call number: QR189 V32
Descriptors: mice, human serum albumin, Freund's complete adjuvant, humoral and cellular immune responses, intraperitoneal immunizations.
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NAL call number: 470 Sci2
Descriptors: antibody formation, natural antigens, Freund's adjuvant, mice, Inbred CBA, cultured tumor cells.

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NAL call number: QR189 V32
Descriptors: human malaria vaccine, Th1 and Th2 subset cytokines, therapeutic use, mice, Inbred BALB/c, C57BL, saponins, T-Lymphocytes.
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Descriptors: sexually transmitted diseases, nontoxic mutant LTK63, IgG, IgA, intranasal immunization.
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NAL call number: 500 N21P
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NAL call number: QR180 A5
Descriptors: DL-PLG microspheres, minimize inflammation, polymeric particulate adjuvants.
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NAL call number: 41.8 Z52
Descriptors: aluminum hydroxide, Freund's adjuvant, immune response, antibody production.
- Ermeling, B.L., E.K. Steffen, R.E. Fish, and R.R. Hook, Jr. (April 1992). **Evaluation of subcutaneous chambers as an alternative to conventional methods of antibody production in chickens.** *Laboratory Animal Science* 42(4):402-407, ISSN:0023-6764.
NAL call number: 410.9 P94
Descriptors: egg yolk, granuloma fluid, Freund's, plastic wiffle balls.

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 Descriptors: pigs, freeze-dried vaccine, Aujeszky's disease, oil emulsion, Levamisol, pseudorabies vaccine.
- European Concerted Action On Macaque Models For Aids Research (1995). **Protection of macaques against simian immunodeficiency virus infection with inactivated vaccines: Comparison of adjuvants, doses and challenge, viruses.** *Vaccine* 13(3):295-300, ISSN:0264-410X.
 NAL call number: QR189 V32
 Descriptors: therapeutic use of adjuvants, formaldehyde, immunization schedule, primates, *Macaca fascicularis*, *Macaca mulatta*.
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 Descriptors: guinea pig, hamster, immunogenicity, Freund's, Ribi, Syntex.
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 NAL call number: QR189 V32
 Descriptors: bacterial, vaccines, diphtheria toxoid.
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 Descriptors: comparison study, commercial adjuvants, Poly-A-poly-U, GERBU, RIBI, Pam3, Specol, Freund's, Titermax, subcutaneous administration, distress.

- Freitas, T.V., C.L. Fortes-Diaz, C.R. Diniz, D.T. Velarde, and C.F. Freitas (1991). **Immunization of horses with *Crotalus durissus terrificus* (South American rattlesnake) venom. A comparison of four different procedures.** *Brazilian Journal of Medical and Biological Research* 24(3):281-290, ISSN: 0100-879X.
NAL call number: R850 A1B72
Descriptors: Freund's complete and incomplete adjuvant, liposomes, aluminum hydroxide, antivenom, ED50, horses.
- Freund, J., J. Casals, and E.P. Himer (1937). **Sensitization and antibody formation after injection of tubercle bacilli and paraffin oil.** *Proceedings of the Society of Experimental Biology and Medicine* 37:509-513, ISSN:0037-9727.
NAL call number: 442.9 S01
Descriptors: guinea pig, rabbit, titer, bacilli, sensitivity.
- Gengoux, C. and C. Leclerc (January 1995). **In vivo induction of CD4+ T cell responses by antigens covalently linked to synthetic microspheres does not require adjuvant.** *International Immunology* 7(1):45-53, ISSN:0953-8178.
Descriptors: hen egg lysozyme, CD4-Positive T-Lymphocytes hybridomas, Inbred BALB/c mice.
- Gillette, R.W. (May 4, 1987). **Alternatives to pristane priming for ascitic fluid and monoclonal antibody production.** *Journal of Immunological Methods* 99(1):21-23, ISSN:0022-1759.
NAL call number: QR180 J6
Descriptors: incomplete Freund's adjuvant, hybridoma cell inoculation, proteose-peptone, thioglycollate, corn oil hybridomas, mice, Inbred BALB/c.
- Giordano, J. and L.V. Rogers (1989). **Peripherally administered serotonin 5-HT₃ receptor antagonists reduce inflammatory pain in rats.** *European Journal of Pharmacology* 170:83-86, ISSN:0014-2999.
NAL call number: QP901 E8
Descriptors: pain model, Freund's complete adjuvant, hind paw injection, drug trials, analgesic tests.
- Glaser, V. (June 1, 1995). **Adjuvants boost safety, efficacy while lowering the costs of new vaccines.** *Genetic Engineering News* 15(11):6-7, ISSN:0270-6377.
NAL call number: QH442 G456
Descriptors: vaccine development, Freund's, aluminum salts, MF59, glycoprotein, Ribi, nonionic surfactant vesicles, saponin Quil A.
- Gonzalez, A., N.D. Greene, and B.D. Murphy (1991). **Active immunization against GnRH in mice and rats: Adjuvant B, an alternative to Freund's adjuvant.** *Journal of Animal Science* 69 (Suppl. 1):396, ISSN:0021-8812.
NAL call number: 49 J82
Descriptors: mice, rats, GnRH immunization, keyhole limpet hemocyanin.

- Goodman, M.G. (1995). **A new approach to vaccine adjuvants. Immunopotential by intracellular T-helper-like signals transmitted by loxoribine.** *Pharmaceutical Biotechnology* 6:581-609, ISSN:1078-0467.
NAL call number: RS380 P53 v.6
Descriptors: loxoribine, B cells, T cells, NK cells, macrophages, and LAK cells, therapeutic use, guanosine, adverse effects.
- Gray, G., R. McGhie, and J.A. Love (October/December 1992). **Antibody production in rabbits: Reducing animal numbers.** *Canadian Association for Laboratory Animal Science Newsletter* 26(4/5):106-110, ISSN: 0045-4354
NAL call number: SF405.5 C36
Descriptors: polyclonal antibodies, carotid cannulation, reduction of animal number.
- Grubhofer, N. (January 1995). **An adjuvant formulation based on N-acetylglucosaminyl-N-acetylmuramyl-L-alanyl-D-isoglutamine with, dimethyldioctadecylammonium chloride and zinc-L-proline complex as synergists.** *Immunology Letters* 44(1):19-24, ISSN:0165-2478.
NAL call number: QR180.I53
Descriptors: mice, Inbred C3H, ternary synergistic composition, ammonium, compounds, Freund's Adjuvant, haptens.
- Gupta, R.K., Jr. P. Griffin, et al. (1996). **The role of adjuvants and delivery systems in modulation of immune response to vaccines.** *Advances in Experimental Medicine and Biology* 397:105-113, ISSN:0065-2598.
NAL call number: QP901.A33
Descriptors: cytokines, antibody isotypes, T helper cell types, aluminum adjuvants, alternative adjuvants.
- Gupta, R.K., B.E. Rost, E. Relyveld, and G.R. Siber (1995). **Adjuvant properties of aluminum and calcium compounds.** *Pharmaceutical Biotechnology* 6:229-248, ISSN:1078-0467.
NAL call number: RS380 P53 v.6
Descriptors: aluminum compounds, eosinophilia, macrophages, calcium compounds.
- Gupta, R.K. and G.R. Siber (October 1995). **Adjuvants for human vaccines: Current status, problems and future prospects.** *Vaccine* 13(14):1263-1276, ISSN:0264-410X.
NAL call number: QR189 V32
Descriptors: synthetic vaccines, toxicity, adverse side-effects, aluminum hydroxide, aluminum phosphate, calcium phosphate and oil emulsions, alternative adjuvants, muramyl dipeptide, monophosphoryl lipid A, liposomes, QS21, MF-59, immunostimulating complexes, biodegradable polymer, microspheres.

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 Descriptors: aluminum compounds, calcium phosphates, bacterial biosynthesis, guinea pigs, mice, IgE, IgG.
- Gursel, M. and G. Gregoriadis (1995). **Immunoadjuvant action of liposomes containing interleukin-2 as a co-adjuvant.** *Proceedings of the Controlled Release Society* 22:572-573, ISSN:1022-0178.
 Descriptors: mice, intramuscular drug administration, interleukin 2, liposome, immunoglobulin G.
- Gustafson, G.L. and M.J. Rhodes (1992). **Bacterial cell wall products as adjuvants: early interferon gamma as a marker for adjuvants that enhance protective immunity.** *Research in Immunology* 143(5):483-488, ISSN:0923-2494.
 NAL call number: QR180 A5
 Descriptors: monophosphoryl lipid A, natural killer cells, bacterial immunostimulants, multiple doses, side effects.
- Hanly, W.C. (January 1995). **Freund's adjuvant and alternatives.** *BRL Bulletin* 10(1):1-4.
 NAL call number: IP SG6428
 Descriptors: action, inflammatory response, laboratory hazard, pain, distress.
- Hanly, W. C. (January 1995). **UIC guidelines for the use of adjuvants in animals.** *BRL Bulletin* 10(1):1-4.
 NAL call number: IP SG6428
 Descriptors: Freund's, laboratory hazard, dose, emulsion, injection site.
- Hanly, W.C. (September 1994). **Adjuvants and their use. Part II.** *BRL Bulletin* 9(9):1-4.
 NAL call number: IP SG6428
 Descriptors: alternative adjuvants, montanide ISA, Ribi, Nitrocellulose-adsorbed protein, encapsulated antigens.
- Hanly, W.C. (August 1994). **Adjuvants and their use. Part I.** *BRL Bulletin* 9(8):1-4.
 NAL call number: IP SG6428
 Descriptors: function, Freund's, advantages and disadvantages.
- Hariharan, K., and G. Braslawsky et al. (1995). **The induction of cytotoxic T cells and tumor regression by soluble antigens in adjuvant formulation.** *Eighty-sixth Annual Meeting of the American Association for Cancer Research, Toronto, Ontario, Canada, March 18-22, 1995. Proceedings of the American Association for Cancer Research Annual Meeting* 36:491, ISSN:0197-016X.
 Descriptors: meeting abstract, mouse, cytotoxic, t lymphocyte, vaccination, immunotherapy.

- Herbert, J.W. (1968). **The mode of action of mineral-oil emulsion adjuvants on antibody production in mice.** *Immunology* 14:301-318, ISSN:0165-2427.
NAL call number: 448.3 IM6
Descriptors: mouse, water-in-oil emulsion, ovalbumin, mechanisms, titer.
- Hess, H., M.K. Gately, et al. (1996). **High doses of interleukin-12 inhibit the development of joint disease in DBA/1 mice immunized with type II collagen in complete Freund's adjuvant.** *European Journal of Immunology* 26(1):187-191, ISSN:0014-2980.
NAL call number: QR180 E87
Descriptors: collagen-induced arthritis, autoimmune joint disease, complete Freund's adjuvant, IL-12, intraperitoneal drug administration, male, mouse.
- Hibma, M. and J.F. Griffin (March 1992). **The effect of adjuvants on active and passive immunity in pregnant deer and their offspring.** *Veterinary Immunology and Immunopathology* 31(3-4): 279-287, ISSN: 0165-2427.
NAL call number: SF757.2 V38
Descriptors: IgM, IgG, Freund's complete adjuvant, diethylaminoethyl dextran, aluminum hydroxide, morbidity.
- Hilgers, L.A., P.L. Platenburg, et al. (May 1994). **A novel non-mineral oil-based adjuvant. II. Efficacy of a synthetic sulfolipopolysaccharide in a squalane-in-water emulsion in pigs.** *Vaccine* 12(7):661-665, ISSN:0264-410X.
NAL call number: QR189 V32
Descriptors: sulfolipopolysaccharide, squalane-in-water emulsion, mineral-oil-in-water adjuvant, porcine vaccines.
- Hilgers, L.A.T. and H. Snippe (1992). **DDA as an immunological adjuvant.** *Research in Immunology* 143(5):494-503, ISSN:0923-2494.
NAL call number: QR180 A5
Descriptors: dimethyldioctadecylammonium bromide, humoral immune response, cell-mediated immune response, toxicity.
- Hill, S.L., M.G. Landavere, and N.R. Rose (1996). **The adjuvant effect of silicone gel and silicone elastomer particles in rats.** *Current Topics in Microbiology and Immunology* 210:123-137, ISSN:0070-217X.
NAL call number: QR1 C8 no.210
Descriptors: male, Harlan Sprague Dawley rats, silicone oil, silicone elastomer, antigen, bovine serum albumin, inflammatory response, injection site.
- Hillam, R.P., R.P. Tengerdy, and G.L. Brown (September 1974). **Local antibody production against the murine toxin of *Yersina pestis* in a golf ball-induced granuloma.** *Infection and Immunity* 10(1):458-463, ISSN:0019-9567.
NAL call number: QR1 I57
Descriptors: IgG, golf ball, granuloma, titer quantity, transudate.

- Hornung, R.L., D.L. Longo, et al. (1995). **Induction of a CD8+ cytotoxic T lymphocyte response to soluble antigen given together with a novel muramyl dipeptide adjuvant, N-acetyl-D-glucosaminyl-(beta1-4)-N-acetylmuramyl-L-alanyl-Disoglutamine (GMDP).** *Therapeutic Immunology* 2(1):7-14, ISSN:0967-0149
Descriptores: C57Bl/6 mice, muramyl dipeptide, ovalbumin, drug comparison, vaccine.
- Hsia, J., T. Tang, M. Parrott, and K. Rogalla (November 1994). **Augmentation of the immune response to influenza vaccine by acetylsalicylic acid: a clinical trial in a geriatric population.** *Methods and Findings in Experimental and Clinical Pharmacology* 16(9):677-683, ISSN:0379-0355.
Descriptores: oral acetylsalicylic acid (ASA), safe, inexpensive, influenza vaccination, geriatric population, interleukin-2 production.
- Hsieh, D.S.T., W.D. Rhine, and R. Langer (January 1983). **Zero-order controlled-release polymer matrices for micro- and macromolecules.** *Journal of Pharmaceutical Sciences* 72(1):17-22, ISSN:0022-3549.
NAL call number: 396.8 J825
Descriptores: hemisphere, drug delivery, polymer.
- Hui, G.S.N., L.Q. Tam, et al. (1991). **Synthetic low-toxicity muramyl dipeptide and monophosphoryl lipid A replace Freund's complete adjuvant in inducing growth-inhibitory antibodies to the Plasmodium falciparum major merozoite surface protein, gp195.** *Infection and Immunity* 59(5):1585-1591, ISSN:0019-9567.
NAL call number: QR1 I57
Descriptores: rabbit, B30-MDP, lipophilic muramyl dipeptide derivative, LA-15-PH, Freund's complete adjuvant, malaria vaccine, antibody production.
- Hunter R., M. Olsen and S. Buynitzky (April 1991) **Adjuvant activity of non-ionic block copolymers. IV. Effect of molecular weight and formulation on titer and isotype of antibody.** *Vaccine* 9:250-256, ISSN:0264-410X.
NAL call number: QR189 V32
Descriptores: vaccines, female ICR mice, subcutaneous injection, IgG.
- Hunter, R.L. and B. Bennett (1984). **The adjuvant activity of non-ionic block polymer surfactants. II. Antibody formation and inflammation related to the structure of triblock and actablock copolymers.** *Journal of Immunology* 133(6):3167-3175, ISSN:0022-1767.
NAL call number: 448.8 J8232
Descriptores: block copolymers, mouse, polyoxyethylene, polyoxypropylene, adsorptive surface.

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 Descriptors: guinea pig, mice, subcutaneous drug administration, intraperitoneal drug administration, aluminum hydroxide.
- Ishihara, C., M. Miyazawa, J. Nishio, I. Azuma, and B. Chesebro (1992). **Use of low toxicity adjuvants and killed virus to induce protective immunity against the Friend murine leukaemia retrovirus-induced disease.** *Vaccine* 10(5):353-356, ISSN:0264-410X.
 NAL call number: QR189 V32
 Descriptors: mouse, combined adjuvant, mycobacterial cord factor, monophosphoryl lipid A, trehalose dimycolate.
- Jackson L.A. and J.P. Opdebeeck (May 1995). **The effect of various adjuvants on the humoral immune response of sheep and cattle to soluble and membrane midgut antigens of *Boophilus microplus*.** *Veterinary Parasitology* 58(1-2):129-141, ISSN:0304-4017.
 Descriptors: sheep, cattle, cattle tick, *Boophilus microplus*, Quil A, Freund's incomplete adjuvant, aluminium hydroxide, immunological procedures, vaccine, intramuscular drug administration.
- Jennings, V.M. (1995). **Review of selected adjuvants used in antibody production.** *ILAR Journal* 37(3):119-125, ISSN:0018-9960.
 NAL call number: QL55 A1I43
 Descriptors: Freund's, RIBI, TiterMax, pain, distress, granuloma, arthritis.
- Johnson, D.K. (1994). **Adjuvant comparison in rabbits.** In: *Rodents and Rabbits Current Research Issues: Proceedings of a conference sponsored by SCAW and WARDS held in Washington, DC on May 21, 1993* S.M. Niemi, J.S. Venable and H.N. Guttman, eds., Scientist Center for Animal Welfare: Greenbelt Maryland, WARDS: Washington, DC, pp. 77-80.
 NAL call number:SF407 R6R63 1994
 Descriptors: polyclonal antibodies, complete Freund's adjuvant, TiterMax, Ribi, Adjuprime.
- Johnson, D.K. (1994). **Adjuvant comparison in rabbits.** *Science and Animal Care* 5(2):2-4.
 NAL call number: HV4701 S35
 Descriptors: Freund's, TiterMax, Adjuprime, Ribi, Squalen, titers, clinical effects.
- Johnston, B.A., H. Eisen, and D. Fry (January 1991). **An evaluation of several adjuvant emulsion regimens for the production of polyclonal antisera in rabbits.** *Laboratory Animal Science* 41(1): 15-21, ISSN:0023-6764.
 NAL call number: 410.9 P94
 Descriptors: polyclonal antisera, emulsion, complete Freund's, incomplete Freund's, RIBI, Montanide ISA 50, Montanide ISA 70.

- Jones, G.L., L. Spencer, R. Lord, H. Edmundson and A.J. Saul (May-June 1991). **High-titer antisera production using three adjuvants and peptide conjugates derived from malarial surface antigen MSA-2.** *Peptide Research* 4(3):138-141, ISSN:1040-5704.
Descriptors: peptide constructs, alum, Freund's adjuvant.
- Jones, G.L., L. Spencer, R. Lord, and A.J. Saul (1996). **Effect of context and adjuvant on the immunogenicity of recombinant proteins and peptide conjugates derived from the polymorphic malarial surface antigen MSA2.** *Vaccine* 14(1):77-84, ISSN:0264-410X.
NAL call number: QR189 V32
Descriptors: Quackenbush mice, Freund's adjuvant, Alum, Algamulin, monoclonal antibodies, recombinant vaccines, immunization, parasites, *Plasmodium falciparum*, protozoa.
- Kato, H., M. Shibano, et al. (1994). **Relationship between hemolytic activity and adsorption capacity of aluminum hydroxide and calcium phosphate as immunological adjuvants for biologicals.** *Microbiology and Immunology* 38(7):543-548, ISSN:0385-5600.
NAL call number: 448.8 J273
Descriptors: guinea pig, vaccine, aluminum hydroxide, calcium phosphate, immunological adjuvant, hemolytic activity.
- Katz, D., S. Lehrer, O. Galan, B.E. Lachmi and S. Cohen (November 1991). **Adjuvant effects of dimethyl dioctadecyl ammonium bromide, complete Freund's adjuvant and aluminium hydroxide on neutralizing antibody, antibody-isotope and delayed-type hypersensitivity responses to Semliki Forest virus in mice.** *FEMS Microbiology Immunology* 3(6):305-320, ISSN:0920-8534.
NAL call number: QR1 F44
Descriptors: adsorption, comparative study, immunoglobulin isotopes.
- Katz, D., Inbar, I., Samina, I., Peleg, B.A., Heller, D.E. (December 1993). **Comparison of dimethyl dioctadecyl ammonium bromide, Freund's complete adjuvant and mineral oil for induction of humoral antibodies, cellular immunity and resistance to Newcastle disease virus in chickens.** *FEMS Immunology and Medical Microbiology* 7(4):303-313, ISSN:0928-8244.
NAL call number: QR180.F46
Descriptors: lipophilic quaternary amine, Newcastle disease, immune responses, Freund's complete adjuvant.
- Ke, Y., R.L. Hunter, and J.A. Kapp (1995). **Induction of humoral and cytolytic responses by ovalbumin in TiterMax and a new synthetic copolymer adjuvant.** *Vaccine Research* 4(1):29-45, ISSN:1056-7909.
Descriptors: mice, TiterMax adjuvant, nonionic block copolymer, ovalbumin-P1005, adjuvant, alternatives, complete Freund's adjuvant, specific humoral and cell-mediated immune response, vaccine.

- Kellner, J., M. Erhard, I. Schraner, and U. Lösch (January 1992). **The influence of various adjuvants on antibody synthesis following immunization with a hapten.** *Biological Chemistry Hoppe-Seyler* 373:51-55, ISSN:0177-3593.
NAL call number: 384 738
Descriptors: Freund's complete adjuvant, synthetic lipopeptides, aluminium hydroxide, mouse, immunostimulation.
- Kim, C.K. and E.J. Jeong (1995). **Development of dried liposome as effective immuno-adjuvant for hepatitis B surface antigen.** *International Journal of Pharmaceutics* 115(2):193-199, ISSN:0378-5173.
Descriptors: hepatitis B surface antigen, trehalose, drug stability, dehydration, rehydration, particle size, immunogenicity.
- Kleinau, S., H. Erlandsson, R. Holmdahl, L. Klareskog (December 1991). **Adjuvant oils induce arthritis in the DA rat. I. Characterization of the disease and evidence for an immunological involvement.** *Journal of Autoimmunology* 4(6):871-880, ISSN:0896-8411.
Descriptors: Freund's incomplete adjuvant, erosive polyarthritis, Lewis rats, mineral oil, Arlacel A, Pristane oil.
- Kleinman, N.R., A.B. Kier, E. Diaconu and J.H. Lass (August 1993). **Posterior paresis induced by Freund's adjuvant in guinea pigs.** *Laboratory Animal Science* 43(4):364-366, ISSN:0023-6764.
NAL call number: 410.9 P94
Descriptors: pain, inadvertent injection, paraspinal muscles.
- Krzystyniak, K., E. Kozłowska, et al. (1995). **Different T-cell activation by streptozotocin and Freund's adjuvant in popliteal lymph node (PLN.).** *International Journal of Immunopharmacology* 17(3):189-196, ISSN:0192-0561.
NAL call number: QR180.I52
Descriptors: mouse, autoimmunity-inducing drugs, Freund's complete adjuvant (FCA), footpad, BALB/c mice, subcutaneous drug administration, drug comparison, T lymphocyte.
- Lachman, L.B., L. Shih, et al. (1995). **Cytokine-containing liposomes as adjuvants for HIV subunit vaccines.** *AIDS Research and Human Retroviruses* 11(8):921-932, ISSN:0889-2229.
Descriptors: mouse, human immunodeficiency virus vaccine, liposome, gamma interferon, interleukin 6, delayed hypersensitivity, humoral immunity, antibody titer.

- Lacour, F. (1985). **Polyadenylic-polyuridylic acid: biological response modifying activities in mice. In vivo organ distribution and pharmacokinetics in rabbits.** *Journal of Biological Response Modifiers* 4:490-494, ISSN:0732-6580.
 Descriptors: poly(A)-poly(U), nontoxic, interferon, breast cancer, humoral and cell-mediated immune responses.
- Lacy, M.J. and E.W. Voss, Jr. (1986). **A modified method to induce immune polyclonal ascites fluid in BALB/c mice using Sp2/0-Ag14 cells.** *Journal of Immunological Methods* 87:169-177, ISSN:0022-1759.
 NAL call number: QR180 J6
 Descriptors: mouse, ascites fluid, Freund's complete adjuvant, Pristane, titer.
- Langer, R. (1981). **Polymers for the sustained release of macromolecules: Their use in a single-step method of immunization.** *Methods in Enzymology* 73:57-75, ISSN:0076-6879.
 NAL call number: QP601 M49
 Descriptors: drug delivery, release kinetics, immunization.
- Langer, R., L. Brown, and E. Edelman (1985). **Controlled release and magnetically modulated release systems for macromolecules.** *Methods in Enzymology* 112:399-423, ISSN:0076-6879.
 NAL call number: QP601 M49
 Descriptors: polymeric delivery system, biocompatibility, single-step immunizations, polypeptides.
- Langhein, C. and J.F. Newman (November 1987). **Antibody response to bacterial antigens covalently bound to biodegradable polymerized serum albumin beads.** *Journal Applied Bacteriology* 63(5):443-448, ISSN: 0021-8847.
 NAL call number: 448.39 So12
 Descriptors: vaccines, *Clostridium botulinum* type D toxin, *Klebsiella pneumoniae*, capsular polysaccharide antigen, rabbit serum, albumin beads, alternative antibody production method.
- Larson, A.A., D.R. Brown, S. El-Atrash, and M.M. Walser (1986). **Pain threshold changes in adjuvant-induced inflammation: A possible model of chronic pain in the mouse.** *Pharmacology, Biochemistry and Behavior* 24:49-53, ISSN:0091-3057.
 NAL call number: QP901 P4
 Descriptors: Freund's complete adjuvant, footpad or lumbar injections, nociception, inflammation, arthritis.

- Leenaars, P.P., A.M., Hendrikson, M.A. Koedam, and E. Claassen (October 1996). **Evaluation of commercially available alternatives to Freund's Complete Adjuvant in mice.** In: *Second World Congress on Alternatives and Animal Use in the Life Sciences October 20-24, 1996, Utrecht, The Netherlands*, J.B.F. van der Valk and L.F.M. van Zutphen, eds., *Alternatives to Laboratory Animals: ATLA 24*(Special Issue):119, ISSN:0261-1929.
NAL call number: Z7994.L3A5
Descriptors: abstract, Freund's incomplete adjuvant, Specol, Montanide, TiterMax, RIBI.
- Leenaars, P.P., A. M. Hendriksen, et al. (1995). **Comparison of adjuvants for immune potentiating properties and side effects in mice.** *Veterinary Immunology and Immunopathology* 48(1/2):123-138, ISSN:0165-2427.
NAL call number: SF757.2 V38
Descriptors: mice, Freund's complete adjuvant, water-in-oil emulsion (Specol), Lactobacillus, immune-stimulating complexes (ISCOM), saponin, Quil A, intraperitoneal, subcutaneous, dorsal, administration, behavioural studies, pathological lesions, adverse effects.
- Leenaars, P.P., A.M., Hendrikson, A.F. Angulo, M.A. Koedam, and E. Claassen (1994). **Evaluation in rabbits of several types of adjuvant.** In: *Welfare and Science Proceedings of the Fifth Symposium of the Federation of European Laboratory Animal Science Associations, June 8-11, 1993, Brighton, UK* John Bunyan, ed., Royal Society of Medical Press: London, pp. 155-161.
NAL call number: QL55 F43 1993
Descriptors: New Zealand White, Freund's, TiterMax, RIBI, Specol, Quil-A, side effects.
- Leenaars, P.P., A.M., Hendrikson, A.F. Angulo, M.A. Koedam, and E. Claassen (1994). **Evaluation of several adjuvants as alternatives to the use of Freund's adjuvant in rabbits.** *Veterinary Immunology and Immunopathology* 40:225-241, ISSN:0165-2427.
NAL call number: SF757.2 V38
Descriptors: Freund's, TiterMax, RIBI, Specol, titer response, inflammation.
- Linblad, E.B. and J.V. Sparack (1987). **Basic concepts in the application of immunological adjuvants.** *Scandinavian Journal of Laboratory Animal Science* 14:1-13, ISSN:0901-3393.
NAL call number: QL55 S322
Descriptors: animal model, route of administration, adjuvant type.
- Lindsay, D.S., R. Parton, A.C. Wardlaw (November 1994). **Adjuvant effect of pertussis toxin on the production of anti-ovalbumin IgE in mice and lack of direct correlation between PCA and ELISA.** *International Archives of Allergy and Immunology* 105(3):281-288, ISSN:1018-2438.
Descriptors: Ham/ICR mice, pertussis toxin, priming dose.

- Lipman, N.S., L.J. Trudel, J.C. Murphy, and Y. Sahali (April 1992). **Comparison of immune response potentiation and in vivo inflammatory effects of Freund's and RIBI adjuvants in mice.** *Laboratory Animal Science* 42(2):193-197, ISSN:0023-6764.
NAL call number: 410.9 P94
Descriptors: Freund's, RIBI, mouse, inflammation, toxicity, bovine gamma globulin.
- Lofthouse, S.A., A.E. Andrews, et al. (August 1995). **Humoral and cellular responses induced by intradermally administered, cytokine and conventional adjuvants.** *Vaccine* 13(12):1131-1137, ISSN:0264-410X.
NAL call number: QR189 V32
Descriptors: sheep, avidin muramyl dipeptide, aluminium hydroxide gel(alum), avidin, Interleukin-1, recombinant proteins, saponins, Quil A, intradermal drug administration, drug comparison.
- Lygren, I., P.G. Burhol, and H.L. Waldum (January-March 1984). **Production and evaluation of VIP antibodies in rabbits.** *Materia Medica Polona* 1(51):26-29, ISSN:0025-5246.
NAL call number: R5 M3
Descriptors: New Zealand rabbits, Freund's adjuvant, vasoactive intestinal polypeptide, subcutaneous injections.
- Ma, J., P.A. Bulger, et al. (August 1994). **Impact of the saponin adjuvant QS-21 and aluminium hydroxide on the immunogenicity of recombinant OspA and OspB of *Borrelia burgdorferi*.** *Vaccine* 12(10):925-932, ISSN:0264-410X.
NAL call number: QR189 V32
Descriptors: mice, Inbred C3H, saponin adjuvant QS-21, aluminium hydroxide, *Borrelia burgdorferi*.
- Mallon, F.M., M.E. Graichen, B.R. Conway, M.S. Landi, and H.C. Hughes (September 1991). **Comparison of antibody response by use of synthetic adjuvant system and Freund complete adjuvant in rabbits.** *American Journal of Veterinary Research* 52(9):1503-1506, ISSN:0002-9645.
NAL call number: 41.8 AM3A
Descriptors: trehalose dimycolate, monophosphoryl lipid A, rabbit, adverse effects, titers.
- Masihi, K.N., W. Brehmer, W. Lange, E. Ribi, S. Schwartzman (1984). **Protective effect of muramyl dipeptide analogs in combination with trehalose dimycolate against aerogenic influenza virus and *Mycobacterium tuberculosis* infections in mice.** *Journal of Biological Response Modifiers* 3:663-671, ISSN:0732-6580.
Descriptors: MDP, antimacrophagic agents, chemiluminescence.

- McClimon, L.B., Glick, B., Dick, J.W. (April/June 1994). **Effect of three commercially available adjuvants on the production of antibodies to *Pasteurella multocida* in broilers.** *Avian Diseases* 38(2):354-357, ISSN:0005-2086.
NAL call number: 41.8 Av5
Descriptors: poultry, incomplete Freund's, Ribí, Hunter's Titermax, vaccine.
- McMurtry, J.P. and N.C. Steele (1989). **Development of an avian calcitonin radioimmunoassay using synthetic chicken calcitonin as immunogen.** *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* 94(B):49-51, ISSN:0305-0491.
NAL call number: QP501.C6
Descriptors: Freund's complete adjuvant, RIBI, calcitonin antibodies, guinea pigs, intradermal injections.
- Mears, G.J. (1995). **Effect of adjuvant on somatostatin (SRIF). antibody production in calves.** *Canadian Journal of Animal Science* 75(3):473-475, ISSN:0008-3984.
NAL call number: 41.8 C163
Descriptors: Freund's adjuvant, alhydrogel, havlogen adjuvant, antibody titers, cattle, dairy industry, pharmaceuticals.
- Michelson, A.M. F. Lacour, and J. Lacour (1985). **Polyadenylic Polyuridylic Acid in the Cotreatment of Cancer.** *Proceedings of the Society for Experimental Biology and Medicine* 179:1-8.
Descriptors: Poly(A).Poly(U), adjuvant, nontoxic, interferon inducer, breast cancer.
- Miettinen, A. (July 1982). **Nephritogenic antibodies against kidney brush border glycoproteins in rabbits injected with Freund's adjuvant.** *Laboratory Investigation* 47(1):67-75, ISSN:0023-6837.
NAL call number: 448.8 L11
Descriptors: Freund's adjuvant, rabbits, tubular brush border antigens, rat kidney, Heymann nephritis.
- Millet, P., K.K. Grady, et al. (April 1995). **Use of the rhesus monkey as an experimental model to test the degree of efficacy of an anti-sporozoite peptide malaria vaccine candidate combined with copolymer-based adjuvants.** *American Journal of Tropical Medicine and Hygiene* 52(4):328-335, ISSN:0002-9637.
NAL call number: 448.8 Am326
Descriptors: humoral response, synthetic peptide carrier protein, diphtheria toxoid, adverse effects, peptide, fragments.
- Mohaghehpour, N., M. Dawson, et al.(1995). **Glucans as immunological adjuvants.** *Advances in Experimental Medicine and Biology* 383(13-22), ISSN:0065-2598.
NAL call number: QP901 A33
Descriptors: rabbits, mice, tissue, interleukin 2, virus protein antibody titer, immunization, humoral immunity, lymphocyte proliferation.

- Mordelet-Dambrine, M., G. Stanislas-Leguern, et al. (December 1994). **Respiratory epithelial permeability in complete Freund's adjuvant rat lung granulomatosis.** *American Journal of Respiratory and Critical Care Medicine* 150(6 Part 1):1660-1666, ISSN:1073-449X.
NAL call number: QR180 C5
Descriptors: Wistar, rats, respiratory epithelium clearance, chemically induced granuloma.
- Mukerjee, R. and U.C. Chaturvedi (December 1995). **Effect of adjuvants on immunization with dengue virus-induced cytotoxic factor.** *Clinical and Experimental Immunology* 102(3):496-500, ISSN:0009-9104.
Descriptors: Freund's incomplete adjuvant, antibody titers, tetanus toxoid, cytokines.
- Murphey-Corb, M., S. Ohkawa, et al. (1994). **Comparative efficacy of a whole killed SIV vaccine in combination with various adjuvants.** *AIDS Research and Human Retroviruses* 10(Suppl. 2): S115, ISSN:0889-2229.
Descriptors: rhesus monkey, simian immunodeficiency virus, immune response.
- Naim, J.O., K.M. Ippolito, and R.J. Lanzafame (March 1995). **The effect of molecular weight and gel preparation on humoral adjuvancy of silicone oils and silicone gels.** *Immunological Investigations* 24(3):537-547, ISSN:0882-0139.
NAL call number: QR180 I452
Descriptors: rats, breast implants, dimethylpolysiloxan, octamethylcyclotetrasiloxane, silicone gel, immunoglobulins.
- Nakaoka, R., Y. Tabata, and Y. Ikada (1995). **Potentiality of gelatin microsphere as immunological adjuvant.** *Vaccine* 13(7):653-661, ISSN: 0264-410X.
NAL call number: QR189 V32
Descriptors: mouse, antibody production, Freund's incomplete adjuvant (FIA), microsphere, phagocytosis, human immunoglobulin.
- Nakashima, S. and H. Kamikawa (1984). **Accelerated expansion of antibody heterogeneity by complete Freund's adjuvant during the response to bacterial amylase.** *Immunology* 53:837-845, ISSN:0019-2805.
NAL call number: 448.3 IM6
Descriptors: mouse, Freund's, optimal dose, isoelectric focusing, antibody heterogeneity.
- Neimi, S.M., J.G. Fox, L.R. Brown, and R. Langer (December 1985). **Evaluation of ethylene-vinyl acetate copolymer as a non-inflammatory alternative to Freund's Complete Adjuvant in rabbits.** *Laboratory Animal Science* 35(6): 609-612, ISSN:0023-6764.
NAL call number: 410.9 P94
Descriptors: antigen delivery device, rabbits, pellet, irritation.

- Nellore, R.V., P.G. Pande, et al. (September-October 1992). **Evaluation of biodegradable microspheres as vaccine adjuvant for hepatitis B surface antigen.** *Journal of Parenteral Science and Technology* 46(5):176-180, ISSN:0279-7976.
 Descriptors: polyglycolic acid, immunostimulants, muramyl dipeptide, aluminum hydroxide, hepatitis B vaccine.
- Nicklas, W. (October 1996). **Alternatives to Freund's adjuvant.** In: *Second World Congress on Alternatives and Animal Use in the Life Sciences October 20-24, 1996, Utrecht, The Netherlands*, J.B.F. van der Valk and L.F.M. van Zutphen, eds., *Alternatives to Laboratory Animals: ATLA* 24(Special Issue):182, ISSN:0261-1929.
 NAL call number: Z7994.L3A5
 Descriptors: abstract, lipid A, muramyl dipeptide, trehalose dimycolate, bacteria, synthetic nonionic block copolymers.
- Nicklas, W. (1992). **Aluminum salts.** *Research in Immunology* 143(5):489-494, ISSN:0923-2494.
 NAL call number: QR180 A5
 Descriptors: combination with other adjuvants, human use, alumen, granulomas.
- Nilsson, B.O. and A. Larsson (1992). **Inert carriers for immunization.** *Research in Immunology* 143(5):553-557, ISSN:0923-2494.
 NAL call number: QR180 A5
 Descriptors: non-Freund's, polyclonal or monoclonal antibodies, soluble immunogens, membranes, beads.
- Olson, M.E. (April 1994). **Vaccination and adjuvants.** *Canadian Association for Laboratory Animal Science Newsletter* 28(2):42-43, ISSN: 0045-4354
 NAL call number: SF405.5 C36
 Descriptors: oil emulsion, mineral adjuvant, bacterial product, other adjuvants, adjuvant free immunization.
- Osebold, J.W. (1982). **Mechanisms of action by immunologic adjuvants.** *Journal of the American Veterinary Medical Association* 181:983-987, ISSN:0003-1488.
 NAL call number: 41.8 AM3
 Descriptors: macrophages, adjuvant, delivery method, cellular response, clinical application.
- Ott, G., G.L. Barchfeld, et al. (November 1995). **Enhancement of humoral response against human influenza vaccine with the simple submicron oil/water emulsion adjuvant MF59.** *Vaccine* 13(16):1557-1562, ISSN:0264-410X.
 NAL call number: QR189 V32
 Descriptors: squalene/H₂O emulsions, muramyl peptide MTP-PE, sorbitan oleate surfactants, low toxicity components, guinea pigs, hamsters.

- Park, S.J., W.H. Kim, et al. (June 1995). **Adjuvant effect of polyadenylic polyuridylic acid on antibody production of recombinant hepatitis B surface antigen in mice.** *International Journal of Immunopharmacology* 17(6):513-516, ISSN:0192-0561.
NAL call number: QR180.I52
Descriptors: mice, antibody production, recombinant hepatitis B surface antigen, adult BALB/c mice, adjuvants, therapeutic use, Inbred BALB/c.
- Perdigon, G., S. Alvarez, et al. (1995). **Comparative effect of the adjuvant capacity of *Lactobacillus casei* and lipopolysaccharide on the intestinal secretory antibody response and resistance to Salmonella infection in mice.** *Food and Agricultural Immunology* 7(3):283-294, ISSN:0954-0105.
NAL call number: QR183.6 F66
Descriptors: mice, comparative study, *Lactobacillus casei*, lipopolysaccharide, oral adjuvants, mucosal immunity.
- Powell, M.F., D.J. Eastman, et al. (February 1995). **Effect of adjuvants on immunogenicity of MN recombinant glycoprotein 120 in guinea pigs.** *AIDS Research and Human Retroviruses* 11(2):203-209, ISSN:0889-2229.
Descriptors: guinea pigs, Freund's adjuvant, alum, QS-21, antibody titers, HIV-1 vaccines.
- Powers, D.C., P.J. Hanscome, and P.J. Pietrobon (October 1995). **In previously immunized elderly adults inactivated influenza A (H1N1) virus vaccines induce poor antibody responses that are not enhanced by liposome adjuvant.** *Vaccine* 13(14):1330-1335, ISSN:0264-410X.
NAL call number: QR189 V32
Descriptors: comparative study, human, adjuvants, vaccine, liposomes.
- Preis, I. and R.S. Langer (1979). **A single-step immunization by sustained antigen release.** *Journal of Immunological Methods* 28:193-197, ISSN:0022-1759.
NAL call number: QR180 J6
Descriptors: mice, polymer pellet, implant, biocompatible.
- Pruett, J.H. and P. Stromberg (May 1995). **Effects of adjuvants on bovine humoral and cellular responses to hypodermin A.** *Veterinary Parasitology* 58(1-2):143-153, ISSN:0304-4017.
Descriptors: serine protease, cattle grub, *Hypoderma lineatum*, complete Freund's adjuvant, delayed hypersensitivity.
- Rehmani, S.F. and P.B. Spradbrow (September 1995). **The influence of adjuvants on oral vaccination of chickens against Newcastle disease.** *Veterinary Microbiology* 46(1-3):63-68, ISSN:0378-1135.
NAL call number: SF601 V44
Descriptors: DEAE-dextran, Quil-A, TiterMax, vaccine, iscoms, avridine, Newcastle disease, administration and dosage.

- Rhine, W.D., D.S.T. Hsieh, and R. Langer (1980). **Polymers for sustained macromolecule release: Procedures to fabricate reproducible delivery systems and control release kinetics.** *Journal of Pharmaceutical Sciences* 69(3):265-270, ISSN:0022-3549.
NAL call number: 396.8 J825
Descriptors: drug delivery, polymeric matrix, release kinetics.
- Ribi, E. (1984). **Beneficial modification of the endotoxin molecule.** *Journal of Biological Response Modifiers* 3:1-9, ISSN:0732-6580.
Descriptors: endotoxin, toxic, lipid A, drug delivery.
- Ribi, E., J. Central, and K. Tacoma (March 1985). **A new immunomodulator with potential clinical applications: Monophosphoryl Lipid A, a detoxified endotoxin.** *Clinical Immunology Newsletter* 6:33-36, ISSN: 0197-1859.
Descriptors: toxic lipid A, endotoxin, mycobacterial adjuvants.
- Richens, E.R., M.F. Tunekar, and K. Behbehani (1987). **Complete Freund's adjuvant has a differential amplification action on the induction of diabetes by streptozotocin in various murine strains: CFA amplifies STZ in murine diabetes.** *Pathology* 19:351-357, ISSN:0031-3025.
Descriptors: CFA, mice, glycemia, insulinitis, diabetes, STZ, pancreatic perilobular inflammation.
- Roberts, M., A. Bacon, et al. (June 1995). **A mutant pertussis toxin molecule that lacks ADP-ribosyltransferase activity, PT-9K/129G, is an effective mucosal adjuvant for intranasally delivered proteins.** *Infection and Immunity* 63(6):2100-2108, ISSN:0019-9567
NAL call number: QR1.I57
Descriptors: genetically detoxified pertussis toxin, tetanus vaccine, intranasally administered, nontoxic C-terminal 50-kDa, Inbred BALB/c mice.
- Robuccio, J.A., J.W. Griffith, et al. (August 1995). **Comparison of the effects of five adjuvants on the antibody response to influenza virus antigen in guinea pigs.** *Laboratory Animal Science* 45(4):420-426, ISSN:0023-6764.
NAL call number: 410.9 P94
Descriptors: Syntex adjuvant, RIBI's adjuvant, TiterMax, adjuvant, Freund's adjuvant, aluminum phosphate adjuvant, tissue, swelling, guinea pigs, adverse effects.
- Ronnberg, B., Fekadu, M. and B. Morein (1995). **Adjuvant activity of non-toxic *Quillaja saponaria* Molina components for use in ISCOM matrix.** *Vaccine* 13(14):1375-1382, ISSN:0264-410X.
NAL call number: QR189 V32
Descriptors: mice, toxicity, adjuvant activity, site of injection, human use, immunological procedures, subcutaneous drug administration.

Ruiloba de Leon, S., J. Gonzalez-Y-Merchand, et al. (1989). **Adjuvant activity of the capsular polysaccharide of *Klebsiella-pneumoniae* K8 on the immune response to the tetanus toxoid vaccine.** *Revista Latinoamericana De Microbiologia* 31(4):335-340, ISSN:0187-4640.

NAL call number: 448.8 R32

Descriptors: antibody production, capsular polysaccharide, aluminum hydroxide.

Sah, H., R. Toddywala, and Y.W. Chien (1995). **Continuous release of proteins from biodegradable microcapsules and in vivo evaluation of their potential as a vaccine adjuvant.** *Journal of Controlled Release* 35(2-3):137-144, ISSN:0168-3659.

NAL call number: RS201 C64J68

Descriptors: mouse, Freund's incomplete adjuvant, lactic/glycolic acid polymers, bovine serum albumin (BSA), aluminum hydroxide, alternative to multiple injections of antigen.

Sarkar, A.K. and M.K. Das (1984). **Preparation of anti-polysaccharide antiserum using liposomes as immunological adjuvant.** *Immunology Letters* 8:197-200, ISSN:0165-2478.

NAL call number: QR180 I53

Descriptors: lecithin, rabbit, IgM, IgG, dextran, Freund's.

Schultz, N., R. Oratz, et al. (April 1995) **Effect of DETOX as an adjuvant for melanoma vaccine.** *Vaccine* 13(5):503-508, ISSN:0264-410X.

NAL call number: QR189 V32

Descriptors: cell wall skeleton, therapeutic use, alum compounds, aluminum sulfate.

Schwarzkopf, C. and B. Thiele (October 1996). **Chicken IGY: Efficacy of alternative adjuvants compared to FCA.** In: *Second World Congress on Alternatives and Animal Use in the Life Sciences October 20-24, 1996, Utrecht, The Netherlands*, J.B.F. van der Valk and L.F.M. van Zutphen, eds., *Alternatives to Laboratory Animals: ATLA* 24(Special Issue):1120, ISSN:0261-1929.

NAL call number: Z7994.L3A5

Descriptors: immunized laying hens, egg yolk, adverse side effects, ABM system, Gerbu adjuvant, TiterMax, lipopeptide Pam-3-Cys, Specol.

Schwarzkopf, C. and B. Thiele (1996). **Effectivity of alternative adjuvants in comparison to Freund's complete adjuvant.** *Alternativen zu Tierexperimenten: ALTEX* 13 (Supplement 96):22-25, ISSN: 0946-7785.

Descriptors: TiterMax, ABM-N, ABM-S, Gerbu, White Leghorn hens, chinchilla bastard rabbits.

- Sedlik C., R. Perraut, et al. (1996). **Antigens linked to synthetic microspheres induce immune responses in primates in the absence of adjuvant.** *Immunobiology* 195(1):105-118, ISSN:0171-2985.
NAL call number: QR180.Z4
Descriptors: mice, synthetic microspheres, *Saimiri sciureus*, immunoglobulins, new antigen formulation, immunological adjuvant, antibody production.
- Shahin, R., M. Leef, et al. (1995). **Adjuvanticity and protective immunity elicited by *Bordetella pertussis* antigens encapsulated in poly(DL-lactide-co-glycolide) microspheres.** *Infection and Immunity* 63(4):1195-1200, ISSN:0019-9567.
NAL call number: QR1.I57
Descriptors: mouse, *Bordetella pertussis* antigens, biodegradable, poly(DL-lactide-co-glycolide) microspheres, parenteral, intranasal, subcutaneous, drug administration, antibody specificity.
- Shahum, E. and H.M. Therien (January 1995). **Liposomal adjuvanticity: effect of encapsulation and surface-linkage on antibody production and proliferative response.** *International Journal of Immunopharmacology* 17(1):9-20, ISSN:0192-0561.
NAL call number: QR180.I52
Descriptors: liposomal antigens, conalbumin, immunoglobulin production, lymphokine production, intraperitoneal, intravenous, and subcutaneous injections, vitro versus vivo, mice.
- Shehadeh, N., F. Calcinaro, et al. (March 19, 1994). **Effect of adjuvant therapy on development of diabetes in mouse and man.** *Lancet* (England) 343(8899):706-707, ISSN:0140-6736.
NAL call number: 448.8 L22
Descriptors: mice, Inbred NOD, type 1 diabetes, Freund's complete adjuvant, vaccine.
- Siegel, R.A. and R. Langer (1984). **Controlled release of polypeptides and other macromolecules.** *Pharmaceutical Research* 1984:2-10.
Descriptors: polymers, drug delivery, magnetism, bioerosion, release kinetics.
- Skea, D.L. and B.H. Barber (1992). **Adjuvant-independent induction of immune responses by antibody-mediated targeting of protein and peptide antigens.** *Research in Immunology* 143(5):568-572, ISSN:0923-2494.
NAL call number: QR180 A5
Descriptors: monoclonal antibodies, antigen-presenting cells, immunotarget, adjuvant-independent delivery, immunoconjugates, rabbit.

- Smith, D.E., M.E. O'Brien, et al. (December 1992). **The selection of an adjuvant emulsion for polyclonal antibody production using a low-molecular-weight antigen in rabbits.** *Laboratory Animal Science* 42(6):599-601, ISSN:0023-6764.
NAL call number: 410.9 P94
Descriptors: Freund's adjuvant (CFA), incomplete Freund's adjuvant (IFA), RIBI, TiterMax.
- Solange, A. (January 12, 1995). **Potential adjuvants for synthetic vaccines. I. Modulators of macrophage activation.** *Chemistry and Physics of Lipids* 75(1):51-58, ISSN:0009-3084.
NAL call number: QP751 C4
Descriptors: human study, cultured cells, glycopeptidolipids, muramyl dipeptide, gamma interferon.
- Soltysik, S., J.Y. Wu, et al. (1995). **Structure/function studies of QS-21 adjuvant: assessment of triterpene aldehyde and glucuronic acid roles in adjuvant function.** *Vaccine* 13(15):1403-1410, ISSN:0264-410X.
NAL call number: QR189 V32
Descriptors: QS-21 derivatives, T-lymphocytes, Saponins, Triterpenes, glucuronidase, lymphocyte transformation, mice, Inbred C57BL, ovalbumin, cytotoxic drug.
- Srinivas, G.R., C.O. Chichester, et al. (March 1994). **Production of type II collagen specific monoclonal antibodies.** *Immunological Investigations* 23(2):85-98, ISSN:0882-0139.
NAL call number: QR180.I452
Descriptors: mice, collagen immunology, hybridomas, polyreactive antibodies.
- Stahl-Henning, C. and G. Rossi G., et al. (1995). **Protection of macaques against simian immunodeficiency virus infection with inactivated vaccines: Comparison of adjuvants, doses and challenge viruses.** *Vaccine* 13(3):295-300, ISSN:0264-410X.
NAL call number: QR189 V32
Descriptors: inactivated virus, vaccine, immunological adjuvant, simian immunodeficiency virus, acquired immune deficiency, monkey.
- Steiner, J.W., B. Langer, and D.L. Schwartz (1960). **The local and systemic effects of Freund's adjuvant and its fractions.** *Archives of Pathology* 70:424-434.
NAL call number: 448.8 AR26
Descriptors: granuloma, lesions, rabbit, mycobacteria, Freund's complete adjuvant.

- Stewart-Tull, D. (October 1996). **Refinement to the use of adjuvants in the production of polyclonal antibodies.** In: *Second World Congress on Alternatives and Animal Use in the Life Sciences October 20-24, 1996, Utrecht, The Netherlands*, J.B.F. van der Valk and L.F.M. van Zutphen, eds., *Alternatives to Laboratory Animals: ATLA 24*(Special Issue):118, ISSN:0261-1929.
NAL call number: Z7994.L3A5
Descriptors: abstract, high titers, animal species, route of administration, antigen per dose, cytotoxicity.
- Stieneker, F., G. Kersten, et al. (January 1995). **Comparison of 24 different adjuvants for inactivated HIV-2 split whole virus as antigen in mice. Induction of titres of binding antibodies and toxicity of the formulations.** *Vaccine* 13(1):45-53, ISSN:0264-410X.
NAL call number: QR189 V32
Descriptors: humoral immune response, NMRI mouse, toxicity, lethal side-effects, polymethylmethacrylate nanoparticles, aluminum compounds, Freund's complete and incomplete adjuvants, fumed silica (Aerosil).
- Stills, Jr., H.F. and M.Q. Bailey (April 1991). **The use of Freund's complete adjuvant.** *Lab Animal* 20(4):25-30, ISSN:0093-7355.
NAL call number: QL55 A1L33
Descriptors: Freund's, antigens, lesions, injection site, pain, emulsion, distress.
- Sugimoto, M., K. Ohishi, et al. (1995). **Oligomannose-coated liposomes as an adjuvant for the induction of cell-mediated immunity.** *FEBS Letters* 363(1-2):53-56, ISSN:0014-5793.
NAL call number: QR180.I452
Descriptors: ovalbumin-reconstituted liposomes, mice, delayed-type footpad swelling, oligomannose coated liposomes, cell-mediated immunity, subcutaneous drug administration.
- Suzue, K. and R.A. Young (January 15, 1996). **Adjuvant-free hsp70 fusion protein system elicits humoral and cellular immune responses to HIV-1 p24.** *Journal of Immunology* 156(2):873-879, ISSN:0022-1767.
NAL call number: 448.8 J8232
Descriptors: heat shock proteins, bacterial and parasitic pathogens, Mycobacterium tuberculosis, humoral and cellular immunity, vaccines, synthetic immunology, mice, Inbred BALB/c.
- Tagliabue, A., P. Ghiara, and D. Boraschi (1992). **Non-inflammatory peptide fragments of IL1 as safe new-generation adjuvants.** *Research in Immunology* 143(5):563-568, ISSN:0923-2494.
NAL call number: QR180 A5
Descriptors: human use, safe, nontoxic, no inflammation, cytokine.

- Tamura, S., A. Yamanaka, et al. (April 1994). **Synergistic action of cholera toxin B subunit (and *Escherichia coli* heat-labile toxin B subunit). and a trace amount of cholera whole toxin as an adjuvant for nasal influenza vaccine.** *Vaccine* 12(5):419-426, ISSN:0264-410X.
NAL call number: QR189 V32
Descriptors: Balb/c mice, *Escherichia coli* immunology, intranasal vaccine inoculation of Cholera Toxin, IgA, IgG.
- Tamura, M., Y.C. Yoo, et al. (January 1995). **Effects of muramyl dipeptide derivatives as adjuvants on the induction of antibody response to recombinant hepatitis B surface antigen.** *Vaccine* 13 (1):77-82, ISSN:0264-410X.
NAL call number: QR189 V32
Descriptors: mice, Inbred BALB/c, alum, acetylmuramyl alanyl isoglutamine derivatives, immunization, intraperitoneal injections, subcutaneous, recombinant proteins.
- Taylor, C.E. (September 1995). **Cytokines as adjuvants for vaccines: Antigen-specific responses differ, from polyclonal responses.** *Infection and Immunity* 63(9):3241-3244, ISSN:0019-9567.
NAL call number: QR1.I57
Descriptors: antibody isotype, polyclonal response, B lymphocytes, epitopes, cytokines.
- Trauger, R.J., A.E. Daigle, et al. (1995). **Safety and immunogenicity of a gp120-depleted, inactivated HIV-1 immunogen: results of a double-blind, adjuvant controlled trial.** *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology* 10(Suppl. 2):S74-S82, ISSN:1077-9450.
Descriptors: incomplete Freund's adjuvant(IFA), humoral and cell-mediated immune (CMI) responses, lymphocyte transformation, vaccines.
- Turanek, J., M. Toman, et al. (February 1994). **Adjuvant effect of liposomes and adamantylamide dipeptide on antigenicity of entrapped synthetic peptide derived from HIV-1 transmembrane region glycoprotein gp41.** *Immunology Letters* 39(2):157-161, ISSN:0165-2478.
NAL call number: QR180 I53
Descriptors: mouse, adamantylamide dipeptide (AdDP), liposomes, Freund's complete adjuvant, aluminum hydroxide (AL).
- Vaitukaitis, J.L. (1981). **Production of antisera with small doses of immunogen: Multiple intradermal injections.** *Methods in Enzymology* 73:47-52, ISSN:0076-6879.
NAL call number: QP601 M49
Descriptors: high affinity antibody, sensitivity, water-in-oil emulsion, intradermal.

- Verheul, A.F.M. and H. Snippe (1992). **Non-ionic block polymer surfactants as immunological adjuvants.** *Research in Immunology* 143(5):512-519, ISSN: 0923-2494.
NAL call number: QR180 A5
Descriptors: low toxicity, synthetic compounds, haptened proteins, liposomes, vaccine formulation, route of administration, side effects.
- Verma, I., P. Pancholi and G.K. Khuller (1995). **Comparative study of the adjuvanticity of FIA and phosphatidylcholine liposomes for purified mycobacterial RNA.** *Journal of Liposome Research* 5(2): 227-240, ISSN:0898-2104.
Descriptors: mycobacterial antigens, mycobacterium tuberculosis, immune response, mouse, vaccination, liposome.
- Vogel, F.R. (1995). **The role of adjuvants in retroviral vaccines.** *International Journal of Immunopharmacology* 17(2):85-90, ISSN:0192-0561.
NAL call number: QR180.I52
Descriptors: subunit antigens, alum adjuvants, novel adjuvants, detoxified lipid A, emulsions, liposomes, biodegradable microspheres, muramyl peptides, saponins, cytotoxic T-cell.
- Vogel, F.R. and C.R. Alving (1995). **Adjuvants and novel vaccines: Conference summary.** *AIDS Research and Human Retroviruses* 11(10):1277-1278, ISSN:0889-2229.
Descriptors: genetic engineering, immunodeficiency virus vaccine, antibody response, virus particle.
- Von Blomberg-Van der Flier, M., R.J. Scheper, G.H. Boerrigter, L. Polak (1984). **Induction of contact sensitivity to a broad variety of allergens with haptened macrophages.** *Journal of Investigative Dermatology* 83(2):91-95, ISSN:0022-202X.
NAL call number: 448.8 J8292
Descriptors: guinea pigs, ulceration, granuloma, Freund's complete adjuvant alternative.
- Wagland, B.M., S.J. McClure, et al. (1996). **Effect of Freund's adjuvants on guinea pigs infected with, or vaccinated against, *Trichostrongylus colubriformis*.** *International Journal for Parasitology* 26(1):85-90, ISSN:0020-7519.
Descriptors: antibody titres, intraperitoneal injection eosinophilia, gastrointestinal nematodes, trichostrongylus colubriformis, antibody titer.
- Warren, H.S., F.R. Vogel, and L.A. Chedid (1986). **Current states of immunological adjuvants.** *Annual Review of Immunology* 4:369-388, ISSN:0732-0582.
NAL call number: QR180 A55
Descriptors: nonbacterial adjuvants, bacterial adjuvants, monokines, lymphokines, muramyl dipeptides, liposomes, Freund's, aluminum compounds.

- Westbrook, S.L., V. Bundoora, and G.H. McDowell (1994). **Immunization of lambs against somatotropin release inhibiting factor to improve productivity: comparison of adjuvants.** *Australian Journal of Agricultural Research*. 45(8):1693-1700, ISSN: 0004-9409.
NAL call number: 23 Au783
Descriptors: lambs, growth rate, live weight gain, body composition, backfat, wool production.
- Whittington, R.J., B.L. Munday, et al. (1994). **Humoral and peritoneal cell responses of rainbow trout (*Oncorhynchus mykiss*) to ovalbumin, *Vibrio anguillarum* and Freund's complete adjuvant following intraperitoneal and bath immunisation.** *Fish and Shellfish Immunology* 4(7):475-488, ISSN:1050-4648.
NAL call number: QL638.97.F55
Descriptors: humoral immune response, ovalbumin, *Vibrio anguillarum*, intraperitoneal administration, Freund's complete adjuvant.
- Wilkie, B. (1990). **Preparation of antigen for immunization using L-Tyrosine or Di-Tyrosine adjuvants.** In: *Adjuvants: Advances in Technology and Alternatives to Freund's* (Conference Proceedings). The Animal Resources Centre and the Faculty of Graduate Studies and Research, McGill University: Montreal, Quebec. pp. 61.
Descriptors: materials, methods, results, Freund's alternative.
- Woodard, L.F. (May 1989). **Adjuvant activity of water-insoluble surfactants.** *Laboratory Animal Science* 39(3):222-225, ISSN:0023-6764.
NAL call number: 410.9 P94
Descriptors: IgG antibody production, Avridine, Freund's, nonionic surfactant, oil-in-water emulsions.
- Wynne, C.R. (1974). **The evaluation of hydrocortisone antibodies produced in rabbits and sheep. I. Radioimmunoassay.** *Immunology* 26(1):97-113, ISSN:0019-2805.
NAL call number: 448.3 IM6
Descriptors: Freund's adjuvant, hydrocortisone hemisuccinate conjugate, dose response, rabbits, sheep.
- Yokochi, T., Y. Inoue, et al. (1995). **Strong adjuvant action of Klebsiella O3 lipopolysaccharide and its inhibition of systemic anaphylaxis.** *FEMS Immunology and Medical Microbiology* 10(3-4): 181-184, ISSN:0928-8244.
Descriptors: IgM, IgG, *Salmonella Minnesota*, mouse, allergy, inflammatory disease.

Zeidner, N.S. and D.L. Belasco (October 1995). **Gliding bacterial adjuvant stimulates feline cytokines in vitro and antigen-specific IgG in vivo.** *Vaccine* 13(14):1294-1299, ISSN:0264-410X.

NAL call number: QR189 V32

Descriptors: comparative study, cytokines, IgG, helminth, cats, lymphocyte transformation, macrophages, T lymphocytes.

Monoclonal Antibody Production

The Report and Recommendations of ECVAM Workshop 23^{1,2}

Uwe Marx,³ M. Jim Embleton,⁴ René Fischer,⁵ Franz P. Gruber,⁶ Ulrika Hansson,⁷ Joachim Heuer,⁸ Wim A. de Leeuw,⁹ Ton Logtenberg,¹⁰ Wolfram Merz,¹¹ Daniel Portetelle,¹² John-Louis Romette,¹³ and Donald W. Straughan¹⁴

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¹ECVAM- The European Centre for the Validation of Alternative Methods; ²This document represents the agreed report of the participants as individual scientists; ³Institute of Clinical Immunology and Transfusion Medicine, Department of Medical Biotechnology, University of Leipzig, Delitzscher Strasse 141, 04129 Leipzig, Germany; ⁴Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Wilmslow Road, Manchester M20 9BX, UK; ⁵Laboratorium für Biochemie I, ETH-Zentrum, Universitätsstrasse 16, 8092 Zurich, Switzerland; ⁶FFVFF, Biberlinstrasse 5, 8032 Zurich, Switzerland; ⁷Swedish Fund for Research without Animal Experiments, Gamla Huddingevagen 437, 12542 Älvsjö, Sweden; ⁸ZEBET, BgVV, Diedersdorfer Weg 1, 12277 Berlin, Germany; ⁹Department of Animal Experiments, Veterinary Public Health Inspectorate, Ministry of Public Health, Welfare and Sport, 2280 HK Rijswijk, The Netherlands; ¹⁰Department of Immunology, University Hospital Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands; ¹¹INTEGRA Biosciences GmbH, Ruhberg 4, 35463 Fernwald, Germany; ¹²Department of Microbiology, Faculty of Agronomy, 6 avenue Maréchal Juin, 5030 Gembloux, Belgium; ¹³Laboratoire de Génie Cellulaire, Université de la Méditerranée, CESB/ESIL, 163 Avenue de Luminy, 13288 Marseille Cedex 9, France; ¹⁴FRAME, Russell and Burch House, 96-98 North Sherwood Street, Nottingham NG1 4EE, UK

Preface

This is the report of the twenty third of a series of workshops organised by the European Centre for the Validation of Alternative Methods (ECVAM). ECVAM's main goal, as defined in 1993 by its Scientific Advisory Committee, is to promote the scientific and regulatory acceptance of alternative methods which are of importance to the biosciences and which reduce, refine or replace the use of laboratory animals. One of the first priorities set by ECVAM was the implementation of procedures which would enable it to become well-informed about the state-of-the-art of non-animal test development and validation, and the potential for the possible incorporation of alternative tests into regulatory procedures. It was decided that this would be best achieved by the organisation of ECVAM workshops on specific topics, at which small groups of invited experts would review the current status of various types of *in vitro* tests and their potential uses, and make recommendations about the best ways forward (1).

The workshop on Monoclonal Antibody Production was held in Angera, Italy, on 19-22 November 1996, under the chairmanship of Uwe Marx (University of Leipzig, Germany). The aim of the workshop was to evaluate the present status of *in vitro* methods for monoclonal antibody (mAb) production, and to compare the advantages and disadvantages of the *in vitro* methods with those of the traditional *in vivo* (malignant ascites) method. The workshop participants assessed various *in vitro* culture systems for the propagation of hybridoma cells in terms of: a) their antibody production capacity; b) the concentration, yield and quality of the mAbs produced; and c) the capital and running costs for their operation. The participants felt that there are already several scientifically satisfactory *in vitro* methods which are both reasonably and practicably available. As these are of moderate cost, and can be shown to be either better or equal to the ascites production method in terms of antibody quality, they concluded that the *in vivo* production of mAbs is no longer necessary, except in rare cases where it is already approved for clinical applications. Differences between several European countries in national policies and legal controls on ascites production were identified, and recommendations have been made to try to increase the routine use of *in vitro* methods by mAb producers and users. The specific conclusions and recommendations made during the workshop are summarised in the final section of this report.

Introduction

Monoclonal antibodies are antibodies which have a single, selected, specificity and which are continuously secreted by "immortalised" hybridoma cells. A hybridoma is a biologically constructed hybrid between an antibody-producing, mortal, lymphoid cell and a malignant, or "immortal", myeloma cell. Following the discovery of hybridoma technology in 1975 (2), developments in mAb production and in their application over the last two decades have had profound implications not only on medical research, diagnosis and therapy, but also on biology in general. Hybridoma technology represents a significant advance because, in principle, it provides a means for obtaining unlimited supplies of highly specific antibodies.

In the production of mAbs, animals (generally rats or mice) first have to be immunised with the target antigen to obtain mortal antibody-producing cells. The biological construction of hybrids, and the selection of hybridomas which produce antibodies with the desired specificities, are carried out *in vitro*. In the early days of hybridoma technology (the late 1970s), the hybridomas developed *in vitro* were then injected into the peritoneal cavity of an animal so that useful amounts of the desired mAb could be harvested from the ascitic fluid. This procedure was considered necessary at the time, since no efficient large-scale *in vitro* methods were available. By the middle of the 1980s, there were already serious doubts regarding the necessity of such a painful animal procedure. Nevertheless, as a result of its early introduction as part of the hybridoma technology, ascites production of mAbs is now employed worldwide, in spite of the ongoing development of *in vitro* technologies and the growing public pressure to replace or reduce animal experiments. The urgent need for experts to disseminate information and make recommendations about antibody production, taking animal welfare issues into consideration, was recognised by ECVAM in holding a workshop on avian antibodies in March 1996 (3) and, subsequently, in organising this workshop on mAb production.

Hybridoma Technology

There are essentially two stages in the production of mAbs: a) the propagation of antibody-producing lymphoid cells *in vivo* and the selection of antibody-producing hybridoma cells *in vitro*; and b) the *in vitro/in vivo* propagation of selected hybridoma clones. The first stage, the formation and selection of the hybridoma clone, involves the use of one or more animals (except in the rare case when a human mAb is being developed), and is carried out in the following way:

1. The antigen is injected into mice (or rats). The antigen is often injected in combination with an adjuvant, to enhance the immune response, even though the use of adjuvant generally leads to severe side effects.
2. After an appropriate interval (5-21 days), the immunised animals are killed and lymphoid cells (including progenitor antibody-producing cells) are isolated from the spleen.
3. The lymphoid cells are fused with myeloma cells which have been grown *in vitro*.
4. The two original cell types and the newly formed hybrids are cultured in a selective medium, such as HAT (hypoxanthine/aminopterin/thymine) medium, which only allows the hybridoma cells to grow.
5. The supernatant media from the numerous *in vitro* microcultures exhibiting a recognisable growth of hybridomas are screened for secretion of the desired antibody, by using various immunoassay procedures.
6. The selected cells are subcultured *in vitro*, using special cloning procedures to ensure that each *in vitro* culture consists of hybridomas with a single antibody specificity only.
7. Hybridoma cells can be cryopreserved at this stage.

The second stage, the propagation of cloned hybridoma cells, can be accomplished either by continuing to grow the cells *in vitro* or by propagating them *in vivo*, in the form of ascites tumours.

Current Demand for Monoclonal Antibodies

The applications of mAbs are numerous and diverse, and they are extensively used in fundamental research, medicine and biotechnology. At present, four user groups can be identified, according to the amount of antibody required. These are summarised in Figure 1.

User group A: less than 0.1g

Approximately 60% of the mAb users in Europe fall within this group, as do many of the current users of the *in vivo* (ascites) method. Small amounts of antibodies are produced for use in fundamental and applied research, the commercial production of special diagnostic kits for research, and for analytical purposes.

User group B: 0.1-0.5g

This group accounts for approximately 30% of mAb users and encompasses a significant number of people still using the *in vivo* method. Antibodies in these amounts are required for the development and production of a wide range of *in vitro* diagnostic kits and reagents, as well as for evaluating the usefulness of novel therapeutic mAbs in animal experiments.

User group C: 0.5-10g

In this group, which accounts for approximately 10% of mAb users, adoption of the *in vivo* method is comparatively rare. The mAbs produced are used in routine diagnostic procedures and in preclinical evaluation studies. They are usually produced by large biotechnology companies but, during the last few years, the production of these mAbs has increasingly been contracted out to smaller facilities.

User group D: more than 10g

Users in this group, who require mAbs for prophylactic and therapeutic purposes *in vivo*, make up less than 1% of all mAb users in Europe. The mAb production processes they use are first developed and validated by the pharmaceutical industry, and are then submitted to a regulatory body for approval.

The extensive use of the ascites method by groups A and B can be attributed to its supposed economic advantage as well as to a lack of inclination to adopt the new techniques. Most of the mAbs produced by these groups are not used in clinical studies and therefore do not have to comply with the standard requirements for pharmaceutical products. This has led to a lack of awareness in these user groups of the disadvantages of ascites production, such as the potential for infection by animal viruses, and the reduced immunoreactivity of the mAb due to contamination with non-specific animal immunoglobulins.

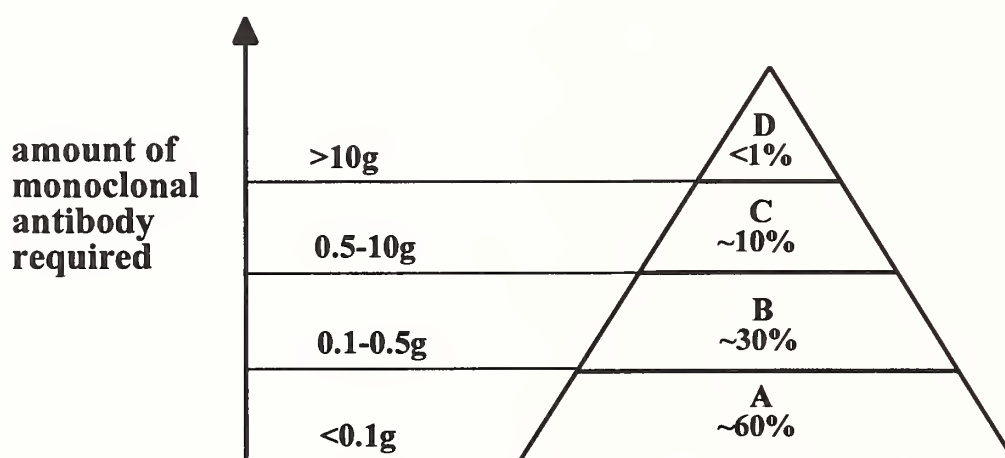


Figure 1. Monoclonal antibody user groups

Monoclonal Antibody Production In Vivo

The *in vivo* procedures entail the use of mice or rats. Initially, the immune systems of the experimental animals are suppressed (1-2 weeks before the intraperitoneal [i.p.] injection of hybridoma cells) by the injection (i.p.) of a primer, such as pristane (2,6,10,14-tetramethylpentadecane) or Freund's incomplete adjuvant. The hybridoma cells then multiply in the peritoneal cavity, and the ascitic fluid which forms is a very rich source of the secreted antibody.

When an adequate amount of ascites has formed, the animal is killed and the ascitic fluid is collected. Sometimes, the ascitic fluid is first "tapped" or drained from the peritoneal cavity while the animal is under anaesthesia, with a second and final harvest being taken once the ascites has reformed. The mAb product can be harvested 5-21 days after the injection of hybridoma cells. Approximately 5ml of ascites can be obtained from a mouse, compared to 10-40ml from a rat. Thus, for the production of a mAb with a given specificity, it may be necessary to use one or more mice, depending on the amount of antibody required.

The main advantage of the ascites method is the extremely high yield of antibody, which generally lies in the range from 1-20mg/ml. In addition, the method is not excessively labour-intensive. However, these advantages are outweighed by a number of disadvantages. The main disadvantage of the ascites method is that it is extremely painful for the animals used, due to the following: a) the injection of primer; b) the resulting peritonitis caused by the primer; c) abdominal tension; and d) the infiltratively-growing tumours which result (4-6). Proper animal husbandry facilities are mandatory. The mAbs produced generally show a reduced immunoreactivity of around 60-70%, as opposed to an immunoreactivity of 90-95% for antibodies produced *in vitro*, due to contamination by biochemically identical immunoglobulins. There is also a potential risk of product contamination by viruses which are pathogenic to humans. A further disadvantage is that the individual batches of harvested ascitic mAb are of variable quality, and they are contaminated with bioreactive cytokines.

In Vitro Production Procedures

In vitro production systems

During the last 20 years, a wide range of *in vitro* production systems have been developed for different purposes. While most of them are useful for the *in vitro* production of mAbs, they differ in terms of: a) the ease with which they are handled; b) the antibody yield per culture or bioreactor run; and c) the maximum antibody titre achievable. The antibodies produced generally express an immunoreactivity of approximately 90-95%, irrespective of the system used.

Three categories of *in vitro* production system can be identified according to the principle underlying the culture system: static and agitated suspension cultures; membrane-based and matrix-based culture systems; and high cell density bioreactors. Some of these systems have been reviewed recently (7, 8).

Static and agitated suspension cultures

Systems in this category, which include the widely used T-flasks, roller cultures and spinner cultures, allow the growth of a maximum of two litres of supernatant per culture unit, and a maximum antibody yield of 100-200mg. They are easy to handle in cell culture laboratories, enable various hybridoma cell lines to be propagated simultaneously, and are useful for most of the users in group A.

Investment costs are low because disposable plasticware is readily available, particularly in the case of T-flasks. The use of serum-free media or low-cost additives enabling a reduction in the serum concentration can greatly reduce costs, while efficiently supporting hybridoma growth (9-13). For example, two serum-free media use a combination of transferrin and insulin (9, 10), whereas two low-serum media use a combination of 1% foetal calf serum (FCS) and 0.1% Primatone, a peptic digest of animal tissues. This supports hybridoma growth in all culture methods tested at least as efficiently as 5% FCS, at approximately 25% of the cost (M.J. Embleton, personal observation).

For the production of mAbs in amounts greater than 100mg, conventional stirred tank bioreactors of different sizes are available. These bioreactors need to be used by specially trained staff and are relevant for user groups A, B and C.

The concentration of hybridoma cells in suspension cultures hardly ever exceeds 5×10^6 cells/ml and, in general, the maximum antibody concentration achievable is below $100 \mu\text{g/ml}$. As a result of the low antibody concentration, the supernatant usually has to be concentrated by ultrafiltration if any further purification steps are to be carried out.

Feeding of cultures may be carried out periodically if required but, in practice, antibody concentration is increased by a factor of 2-4 times if the cultures are allowed to grow to exhaustion over 2-3 weeks without feeding.

Membrane-based and matrix-based culture systems

This category includes membrane-based and matrix-based static cultures as well as suspension bioreactors. These systems are suitable for user groups A, B and C, which require up to 10g of mAb. In membrane-based systems, the cells are cultured in compartments separated from the nutrient supply by perfusion membranes; special gassing membranes enhance the oxygen transfer into these systems. They produce yields of up to 100mg per culture (user group A), and generate intermediate antibody concentrations of up to $500 \mu\text{g/ml}$. In addition, they are easy to handle and enable various different cultures to be run simultaneously in routinely equipped cell culture laboratories.

In matrix-based systems, such as fluidised bed or ceramic bioreactors, the immobilisation of cells on matrices enables them to be perfused actively and continuously with fresh medium. Irrespective of the size and running time of the bioreactors, 0.1-10g of mAbs (user groups B and C) can be produced, corresponding to a maximum concentration of $500 \mu\text{g/ml}$. In most cases, the supernatant produced has to be concentrated by precipitation or ultrafiltration before special purification procedures can be carried out. Special training is required for the proper handling of these systems.

High cell density bioreactors

This category includes all culture systems which are capable of generating cell densities greater than 10^8 cells/ml and which, in certain cases, can maintain tissue-like cultures viable. The bioreactors meet the needs of user groups B and C, as they are capable of generating 100mg - > 10g mAb. The corresponding concentrations lie in the range from 0.5-5mg/ml, due to the high cell densities in these systems. They can be run in conventional cell culture laboratories and models are available for the simultaneous propagation of different cell lines. The product can be used directly or purified without prior concentration. Training is recommended for these systems and is usually provided by the manufacturer.

In the most common system within this category, the hollow fibre bioreactor, the culture medium is passed through bundles of hollow fibres, enabling the cell growth compartment to be perfused continuously and effectively. Due to the high antibody concentration, the maximum amount of 500mg of antibody needed by user group B can be produced in a bulk of only 500ml of supernatant, which is easy to handle and process in a conventional cell culture laboratory. Even for user group C, which requires up to 10g of mAb, the total product can be produced in only 10 litres.

The different categories of culture system are listed according to their usefulness to the different user groups in Table I. Instead of the maximum achievable mAb concentration, the concentration which is normally achievable is given. The types of systems recommended for the different user groups, on the basis of their ease of handling, production costs and advantages with respect to antibody purification, are highlighted in the table.

Table 1. Appropriate culture systems for the four monoclonal antibody user groups

Categories of culture system	User group			
	A	B	C	D
Amount required	≤0.1g (~ 60% of users)	0.1-0.5g (~ 30% of users)	0.5-10g (~ 10% of users)	≥10g (< 1% of users)
Static and agitated suspension cultures	e.g. T-flasks, rollers, spinners	e.g. small scale stirred tank bioreactors, working volume < 10 litres	e.g. small scale stirred tank bioreactors, working volume 10-200 litres	e.g. large scale stirred tank bioreactors
Usual mAb concentration 50µg/ml	V _{max} = 2 litres	V _{max} = 10 litres	V _{max} = 200 litres	
Membrane-based and matrix-based static cultures	e.g. membrane-based static disposable cell culture flasks and rotatable devices, spinner flasks with an incorporated harvest with cell retention	e.g. fluidised bed bioreactors, ceramic matrix-based bioreactors, agitated bioreactors with perfusion and gassing membranes	e.g. fluidised bed bioreactors, ceramic matrix-based bioreactors, agitated bioreactors with perfusion and gassing membranes	
Usual mAb concentration 0.2mg/ml	V _{max} = 0.5 litres	V _{max} = 2.5 litres	V _{max} = 50 litres	
High cell density bioreactors	e.g. miniaturised hollow fibre bioreactors with several culture modules	e.g. small scale hollow fibre bioreactors with several culture modules	e.g. large scale hollow fibre bioreactors	
Usual mAb concentration 1mg/ml	V _{max} = 100ml	V _{max} = 0.5 litres	V _{max} = 10 litres	

V_{max} = maximum volume of supernatant to be processed during purification, assuming the usual antibody concentration for that category.

The most suitable culture systems for the four user groups are shown in bold font.

In vitro process development

Several problems are associated with the use of serum-containing media for the *in vitro* production of mAbs, the most important being the high protein content which makes antibody purification either difficult or impossible. Other problems are animal welfare concerns relating to the production of foetal serum, its cost, its uncontrollable variability in quality from one lot to another, and the risk of its contamination by viruses, mycoplasma and unsuspected prions (14).

All commercial companies with a long experience of cell culture, and many small new biotechnology groups, now offer various serum replacements from bovine plasma and serum substitutes, and ready-to-use serum-free media which may contain many serum-derived proteins ($\sim 3\text{mg/l}$) or reduced amounts of essential proteins ($\sim 30\mu\text{g/ml}$), or which may be devoid of proteins and peptides. Potentially important supplements are also supplied separately to fortify and optimise basal versions of the classical media currently used (15, 16).

Hybridoma growth and mAb production in serum-free media are variable processes which depend on the physical and nutritional requirements of: a) the specific hybridoma cell line; b) the complexity of the serum-free formulation; and c) the culture conditions of the bioreactors (17). Therefore, during the weaning process by which a subpopulation of cells is adapted to growth in a new environment, one needs to optimise criteria such as the cell growth rate, the maximum cell concentration, the final mAb concentration, and the quality of the mAb and its production rate. It is also necessary to ensure that the selected subpopulation exhibits the same immunoreactivity as the population which was cultured in the presence of serum (16).

In most cases, the use of an optimised serum-free formulation rather than a serum-containing medium offers two advantages: a) the mAb is produced in greater yield and with less expense (16, 18); and b) subsequent downstream processing is facilitated.

Monoclonal antibody quality

Both monoclonal and polyclonal immunoglobulin G (IgG) antibodies are N-glycosylated at amino acid 297, a conserved asparagine (Asn) residue in the second constant domain of the heavy chain (CH2). Human serum IgG might be associated with at least 30 different biantennary complex oligosaccharides (19), but these only represent 2-5% of the antibody's molecular weight.

Under physiological conditions, N-glycosylation at Asn 297 plays an important role in several biochemical processes: a) the fixation of complement C1q (17, 20; G. Winter, A.R. Duncan and D. Burton, patent number PCT/GB88/002111); b) the binding of Fc- γ receptors; and c) the resistance of the antibody to proteolysis. In addition, biologically important processes, such as phagocytosis, antigen-dependent cellular cytotoxicity, and the clearance and placental transfer of mAbs, can be influenced by the type, sequence and structure of their glycosylation.

In addition to glycosylation at Asn 297, glycosylation also occurs, in very rare cases, in the variable region of mAbs (21). If such an additional glycosylation is present on a mAb, it may influence its antigen binding capacity, with the result that the respective hybridoma clone is unlikely to be picked out by the initial antigen-specific selection procedures.

Glycosylation is a complex post-translational event which can be influenced by a variety of factors, such as the culture conditions, the protein and carbohydrate supplements in the medium, and the purification procedures. Thus, the *in vitro* methods enable the desired glycosylation

structure to be obtained by making an appropriate choice of these factors. What is often needed, for example, are mAbs with a glycosylation pattern of the biantennary complex oligosaccharide type, with terminal sialic acid residues, and this can be generated in hollow fibre bioreactors. In contrast, when antibodies are produced by the ascites method, it is impossible to influence their glycosylation pattern, which may vary from mouse to mouse.

Generally, the glycosylation issue is only relevant to users who want to use the antibodies *in vivo*, either in humans or in animal experiments (user groups B, C and D), and to users who need to perform experiments on the binding of mAbs to complement proteins or Fc-receptors. In summary, there are no reasonable arguments based on antibody glycosylation which support the use of *in vivo* methods.

Economic aspects

The relative costs of mAb production by *in vitro* methods as opposed to the *in vivo* ascites method has been addressed by several authors recently (22-25). Although many have concluded that the *in vitro* alternatives are comparable in cost to the *in vivo* method, individual calculations have been based on different assumptions. As a consequence of the "out-sourcing" policy which is currently widely adopted by industry and universities, "full cost analyses" have to be made for given technologies. Such analyses reveal a trend in which the costs of mAb production by the ascites method are continually increasing, whereas the costs associated with the various *in vitro* methods are decreasing. The increasing costs of *in vivo* production are largely a result of the increasing costs of laboratory animals.

In contrast, the disposable materials needed for *in vitro* mAb production are decreasing in cost as the production technology improves. The increasing demand for bioreactors is reinforcing this trend by allowing manufacturers to produce them on a larger scale, leading to a reduction in their production costs.

These two cost development curves indicate that there is no driving force which will eventually favour the *in vivo* production of mAbs. The adoption of *in vitro* methods by user groups C and D has led to moderate increases in costs which, at present, are no more than 1.5-3.0 times higher than those associated with the *in vivo* production procedure.

It is desirable that centres of excellence become available for an intermediate period, to help the different user groups adapt their own facilities for mAb production *in vitro*. Such centres of excellence would also be of enormous educational value, by providing training in *in vitro* cell culture technologies.

Advanced technologies and future developments

With novel recombinant DNA-based technologies, such as phage display libraries and direct cloning into plasmids, experimental animals are used solely for the immunisation stage or the need to use animals at all is obviated. The realisation that antibody fragments can be expressed on the surface of bacteriophage particles has revolutionised our ability to mimic B-cell immune systems *in vitro* (26, 27). Very large collections of antibody molecules (libraries) can be expressed on the surface of filamentous bacteriophage particles so that antibodies with desired specificities and high affinities can be obtained from these libraries by affinity selection, by using a wide variety of target

antigens such as recombinant proteins and intact prokaryotic and eukaryotic cells (26-28). Phage display libraries can be constructed from immunoglobulin genes of any species, including humans, and often incorporate synthetic nucleotide sequences. In many cases, sufficiently large repertoires enable the selection of antibodies without prior immunisation of B-cell donors, and this therefore avoids the need to use living animals.

Selected antibody fragments can be recloned into a variety of vectors to produce molecules with tailor-made properties such as whole immunoglobulins of any isotype as well as bivalent or bispecific antibodies. The incorporation of affinity tags enables these recombinant proteins to be rapidly purified after their expression in prokaryotic and eukaryotic expression systems. Importantly, phage antibody display libraries allow the selection of novel specificities against non-immunogenic or unknown target antigens (26). Similarly, large libraries of linear or conformationally constrained small peptides expressed on phage particles enable the selection of even smaller "binding" molecules with desired specificities and affinities (29).

It can be envisaged that, in the near future, binding molecules could be selected from an array of peptide and antibody phage display libraries, and relevant molecules could be produced in *in vitro* expression systems or by peptide synthesis.

Regulatory Aspects

General remarks

Two important laws exist in Europe for the protection of laboratory animals: a) *Council Directive 86/609/EEC* (30); and b) *the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, ETS 123* (31). Both the Directive and the Convention require alternatives to be used when "reasonably and practicably available", but each country is free to adopt stricter measures.

The Directive came into force in 1986; the Convention was opened for signature by the Member Countries of the Council of Europe on 18 March 1986, and came into force in 1991. The 15 Member States of the European Union (EU) are required to incorporate the Directive into their national laws, but the 43 Member Countries of the Council of Europe are not legally obliged to sign the Convention. However, once a Member Country has voluntarily signed and ratified the Convention, it is then required under international law to implement the provisions of the Convention within its territory. So far, the Convention has been both signed and ratified by ten countries, namely: Belgium, Cyprus, Finland, Germany, Greece, The Netherlands, Norway, Spain, Sweden and Switzerland.

National policies and their impact on reducing the use of the ascites method

United Kingdom

The *Animals (Scientific Procedures) Act 1986* (32), which came into force in 1987, effectively implements *Directive 86/609/EEC* in the UK. A project licence issued under the terms of this Act is required for all *in vivo* production of mAbs by the ascites method. Applicants for project

licences are required to justify their proposals in writing, and the Home Secretary (acting on the advice of an expert Inspectorate) then decides whether, and on what terms, to grant the licence.

In December 1991, the UK Home Office issued advice on protocols for minimal severity for raising antibodies using live animals (33). According to this advice, "The malignant ascites method may be justified where less than 20 mice are needed on a one-off basis for a particular mAb. If appropriate facilities for the production of the mAb *in vitro* are available, it is expected that these will be used in preference to the ascites method in mice." The Home Office advice also included recommendations for the use of pristane, for tapping ascites, and on the humane endpoints to be observed when using the malignant ascites method.

The use of animals with hybridomas for mAb production *in vivo* was identified for the first time in the statistics for 1990. From 1990-1994, the number of animals with hybridomas (mainly mice) fell by 51.5%, from 46,188 to 22,391, at a time when total animal use decreased from 3,100,553 (1990) to 2,772,758 (1994). Thus, hybridoma use decreased not only in absolute terms, but also as a percentage of the total number of animals used (including for breeding harmful strains) from 1.49% to 0.83%. Assuming that the total production and use of mAbs did not decrease in the UK over this period, then the statistics are fully compatible with an increasing use of *in vitro* production methods in preference to *in vivo* ones. Indeed, it is known that, by using *in vitro* methods, some large mAb producers have reduced the number of mice used for *in vivo* production by a factor of ten.

It is not yet known whether the Home Office has conducted a review with the following objectives: a) to confirm that all project licence holders in the UK are following the formal advice referred to previously wherever possible; b) to determine the nature, rationale and geographic location for all current use of the ascites method and, in particular, to establish whether such use is routine or exceptional; and c) to discover whether an alleged lack of equipment or expertise for mAb production *in vitro* are accepted as sufficient reasons for allowing the continued use of the ascites method.

Germany

In 1989, a national hearing was held at ZEBET (National Centre for the Documentation and Evaluation of Alternatives to Animal Experiments) to evaluate the current *in vitro* methods for the production of mAbs as replacement alternatives to the ascites mouse procedure (34). The consensus of opinion among national experts was that the production of mAbs *in vivo* should only be permitted in the following exceptional cases: a) when the mAbs are intended for diagnostic and therapeutic purposes in humans, provided that no other options are available; b) when hybridoma cells need to be rescued because they have either failed to grow *in vitro* or they have become infected; and c) when the mAbs are needed to investigate new scientific problems.

Several legal technicalities in connection with these exemptions are noteworthy. Exemption 1 does not breach §7.1 of the *German Animal Protection Act* (Tierschutzgesetz §7.1), since the production of mAbs in this case is not considered to be part of an experimental procedure, and is therefore not considered to be an animal experiment according to this Act. On the other hand, Exemptions 2 and 3 do relate to animal experiments according to §7.1 of the *German Animal Protection Act*, and therefore have to be authorised in accordance with §8.1. Furthermore,

Exemption 2 will only be granted if the mAbs are produced for a specific research project and not for distribution to third parties.

The Netherlands

The *Netherlands Code of Practice for the Production of Monoclonal Antibodies* (4) was issued in 1989 and consists of a small set of guidelines and general information concerning technical matters, pathology, clinical signs and distress in relation to mAb production. Among other things, the guidelines concern: a) the maximum number of mice to be used (5-10) per hybridoma; b) the skill and authorisation of the persons concerned; c) the justification for the protocol; and d) the responsibilities of the day-to-day caretaker, the researcher and the animal welfare officer.

In 1989, the Netherlands Veterinary Public Health Inspectorate, which is empowered to supervise compliance with the provisions of the *Experiments on Animals Act* (1977), issued a *Code of Practice for the Production of Monoclonal Antibodies* (4). The Code was drawn up by a working group established by the Inspectorate. The working group consisted of representatives from five scientific societies: the Netherlands Society for Immunology, the Netherlands Society for Microbiology, the Netherlands Society for Pathology, the Netherlands Society for Infectious Diseases, and the Netherlands Society for Laboratory Animal Science. The Code is not mandatory, but is intended to serve as a tool for researchers, animal welfare officers, biotechnicians and local ethical review committees.

Three years after the Code was issued, an evaluation of its effect led to the following conclusions: a) many institutes were holding discussions on the subject of mAb production, as a result of the Code; b) a number of institutes had changed their institutional policies; c) in several institutes, facilities for *in vitro* production had been established; d) in some institutes, *in vivo* production had been completely replaced by *in vitro* production; e) the total number of animals used for the *in vivo* production of mAbs had been significantly reduced (from more than 10,000 in 1990 to less than 1000 in 1995); f) some institutes were contracting out the *in vitro* production to other institutes; and g) in some institutes, the adoption of *in vitro* production was being hampered by the relative ease of *in vivo* production.

In 1995, a symposium was organised entitled *The Production of Monoclonal Antibodies: Are Animals Still Needed?* (25). There were about 120 participants, who were mainly researchers and animal welfare officers. Several researchers presented their experiences of the *in vitro* production of a large number of mAbs. The Inspectorate used the symposium to investigate whether there was consensus of opinion among the experts concerned. This played a key role in the legislation which followed; Article 10 of the *Netherlands Experiments on Animal Act*, which is the equivalent of Article 7.2 of *Directive 86/609/EEC* (30), states:

"No animal experiment shall be conducted for a purpose which, according to the consensus of opinion among experts, may also be achieved by means other than an experiment on animals, or by means of an experiment using fewer animals or entailing less distress than the experiment in question".

Taking into consideration the discussions and information presented, the Inspectorate decided that Article 10 was fully applicable to the *in vivo* production of mAbs. One month later, on 1

January 1996, a ban on the *in vivo* production came into force. Exemptions could only be granted on the basis of a good scientific justification. By the end of 1996, the Inspectorate had received five requests for exemption.

These results make it clear that the Code of Practice had a substantial effect and created the climate in which a ban could eventually be established. The involvement of researchers and animal welfare officers at all stages in the process appears to have been essential in achieving this ban.

Sweden

Sweden is bound by three different regulations concerning the use of alternative methods: the *European Convention* (31; Article 6), the *Swedish Animal Protection Act* (Section 49:2), and *Directive 86/609/EEC* (30; Article 7). The Swedish law is stricter than the Convention in that it states that existing alternative methods must be used and instructs the animal ethics committees to "advise against the use of animals for such purposes where it is possible to acquire comparable information by other means". This wording does not allow for exemptions, such as for economic reasons, lack of equipment, and/or lack of familiarity with alternative methods on the part of the scientist.

In May 1990, the Swedish National Board for Laboratory Animals issued a general recommendation regarding mAb production (LSFS 1990:21; Subject No. 29) which stated that existing alternative methods should normally be used, but that use of the ascites method may be justified in certain cases, such as for the purification of infected cell lines. When applying to use animals, the experiment director must provide information on other methods which have been tried or considered, so that the ethics review committee can assess whether any difficulties preclude the use of *in vitro* techniques in particular cases. The general recommendation regarding mAb production also includes statements on the distress of animals, as well as advice on the use of pristane, abdominal swelling, the killing of animals, and the tapping of ascites.

The use of animals for the propagation of mAbs by the ascites method is not identified in the national statistics on animal use. The numbers given below are derived from the applications approved by the the seven Swedish animal ethics committees.

In spite of the strict wording of the *Swedish Animal Protection Act*, the Swedish animal ethics committees approved antibody production by the ascites method in more than 1000 animals in both 1994 and 1995. In the majority of cases, the approvals were given without the experiment director having to justify the use of animals. In certain cases, however, the director was advised to follow the recommendations given by the National Board for Laboratory Animals (that is, the section concerning the treatment of animals used for propagation of mAbs).

Switzerland

In 1989, the Swiss Federal Veterinary Office (BVET) informed all scientists that the production of mAbs by the ascites method would become a fundamental breach of Swiss animal welfare legislation as from 1994, and so they had five years to change their methods. The general ban on ascites production was implemented in 1994 by *Animal Welfare Guideline 5.01* (BVET, 20 May 1994). This stated that, in principle, mAbs could be obtained *in vitro*; and that, as a rule, applications for ascites production were to be refused. However, a number of exceptions were

envisaged: a) the development of mAbs for diagnostic and therapeutic purposes in cases of medical emergency; and b) the development of mAbs to rescue single hybridomas when it can be documented that they are not growing *in vitro* satisfactorily or are contaminated.

If exemptions are granted, each animal has to be documented and checked at least once a day. Animals with a weight gain of over 20% have to be killed immediately to harvest the ascites. Although this should usually be drained from dead animals, living animals may also be used, but the authorities have to be notified in every case. In 1996 there was not a single reported instance of exceptional mAb production in ascites mice. However, some scientific groups ordered custom-made mAbs from commercial suppliers outside Switzerland.

In 1993 the Swiss Foundation Research 3R started a validation study on the *in vitro* production of mAbs and provided hollow fibre reactors free of charge to 31 research centres throughout Switzerland. The preliminary results show that 24 groups are still working with mAbs; eight of them have changed to other *in vitro* mAb production systems, mostly with a lower yield (Research Foundation 3R, Switzerland, unpublished). Four groups indicated that the yield obtained with the hollow fibre reactor was insufficient; on average, six mAbs were produced per year by each group, with concentrations ranging from 20-200mg/ml. Of the 24 groups still working with mAbs, 17 thought that universities should provide central mAb production units, and 13 of them felt that this should be done on a no-profit basis. Twenty two groups bought custom-made mAbs in 1995, 16 of them from outside Switzerland. About 80% of the mAbs purchased were produced *in vitro*. Twenty one of the 24 groups welcomed the labelling of commercially available mAbs as either "*in vitro* produced" or "*in vivo* produced". The expected demand per group was 18 mAbs per year, with amounts ranging from 110-1150mg.

Conclusions and Recommendations

The workshop participants noted a number of difficulties which are preventing a complete assessment of the impact and usefulness of *in vitro* methods. There is a lack of information on the extent of *in vivo* production in most EU Member States, due to incomplete statistics on laboratory animal use. Several countries within the EU do not have an effective system for project review or for the justification of animal use, nor do they require explanations of why *in vitro* methods cannot be used. The workshop participants felt that all Member States should collect such information, albeit in summary form, and make it available. They also suggested that mAb manufacturers supply information on how their antibodies are produced, for example, by listing this in their catalogues.

Difficulties also arise from mAbs produced *in vivo* being imported into countries where such *in vivo* production is either prohibited or is only permitted in exceptional cases. Without any restrictions being placed on the importation of such mAbs, it is possible for scientists in countries where guidelines are strictly applied to export hybridoma cell lines to countries with lax policies, so that they can later re-import mAbs which have been produced *in vivo*. In Switzerland, for example, one third of the mAbs which are imported have been produced *in vivo* (René Fischer, unpublished observation). The workshop participants felt that the importation of products obtained by methods which breach existing guidelines, such as *Directive 86/609/EEC* (30) and the *European Convention* (31) cannot be justified.

Many mAb users merely require the antibodies as a tool. Such users may not have the knowledge or experience of relevant *in vitro* methods, so their opinions on the usefulness of *in vitro* methods will not be objective and should be treated with caution. It is desirable that such scientists, and those reviewing their applications, take advice from those with experience in *in vitro* methods and in the supply of products manufactured by such methods.

Article 7.2 of *Directive 86/609/EEC* (30) states that:

“An experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available.”

This is comparable to Article 6.1 of the *Council of Europe Convention* (31). In the light of the above requirement and current knowledge, it was concluded that for all levels of mAb production: a) there are one or more *in vitro* methods which are not only scientifically acceptable but are also reasonably and practicably available; and, as a consequence, b) *in vivo* mAb production can no longer be justified and should cease. However, to enable users time to acquire and implement the new techniques, and for administrative reasons, a transitional period of no more than two years should be allowed, before a complete ban on *in vivo* production is implemented.

Where there is an exceptional need for an emergency therapeutic application, the *in vivo* production of mAbs should continue to be permitted. In those cases where there is an existing regulatory approval for a diagnostic or therapeutic mAb produced by the ascites method, such an *in vivo* method has to be accepted until the approval expires. In addition, the ascites method may be needed in other very exceptional circumstances, where verifiable efforts have failed to produce the mAb *in vitro*. In this situation, each animal experiment should be scientifically justified on a case-by-case basis, and the mAb production should be limited in terms of time and the number of animals to be used. It is also expected that continuing efforts be made to produce the mAb *in vitro*.

The main conclusions and recommendations from this ECVAM workshop on mAb production are summarised below:

1. Various *in vitro* mAb production systems have been developed to meet the needs of a diverse range of users.
2. New recombinant DNA technologies are emerging which enable the expression of designer peptides and proteins, making the ascites method of mAb production redundant.
3. There are differences in the regulations between different European countries, as well as differences in the extent to which they are implemented.
4. The *in vivo* production of mAbs should be prohibited in those countries which are members of the EU and/or have ratified the *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes*.
5. Before a ban on *in vivo* production comes into force, centres of excellence offering advice and, if appropriate, assistance should be established, to help laboratories adapt to the use of *in vitro*

methods. A transitional period of no more than two years should be allowed to enable users time to acquire and implement the new techniques, and for administrative reasons, before such a ban is implemented.

6. Commercially available mAbs should be unambiguously labelled to show whether they were produced *in vivo* or *in vitro*.
7. Ascites-produced mAbs imported into the EU should be labelled to indicate their country of origin.
8. To ensure that *in vivo* mAb production is not performed unnecessarily, there is an urgent need for effective inspection systems, as well as for the resources to implement these, at the level of individual user establishments.
9. Project reviews and inspection systems should be subject to approval. In countries where there is no project review system, one should be introduced. In countries where there is a project review system, it should be considered whether this system meets the necessary approval criteria, especially with respect to the requirement to justify any use of *in vivo* methods.
10. The collection of statistics must be improved in all Member States of the EU, and these should include the numbers and species of animals used for mAb production by the ascites method.
11. In scientific reports, it should be mentioned how the mAbs were produced. Editorial Boards of scientific journals should include this requirement in their *Instructions to Authors*.

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Appendix 1

Proposed European Guideline on Monoclonal Antibody Production

Directive 86/609/EEC and Convention ETS 123

The purpose of this Guideline is to advise Member States on the application to monoclonal antibody (mAb) production of the Three Rs principles enshrined in Article 7 of *Directive 86/609/EEC* (1) and Articles 6, 7 and 8 of the *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, ETS 123* (2), whilst having regard to the right of the Member States to apply or adopt stricter measures (Article 24 of the Directive and Article 4 of the Convention). In particular, this Guideline aims to provide specific advice to scientists and project reviewers on what is currently regarded as best practice by experts in the field.

Article 7.2 of the Directive requires that "an experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available". Also, Article 7.3 of the Directive states that "in a choice between experiments, those which use the minimum number of animals cause the least pain, suffering, distress, and lasting harm and which are most likely to provide satisfactory results shall be selected". While "as a general principle", Article 7.4 of the Directive requires that "all experiments shall be designed to avoid distress and unnecessary pain and suffering to experimental animals". These requirements are also documented in Articles 6.1, 7 and 8a, respectively, of the Convention (2).

Article 2 of the Directive and Article 1 of the Convention cover any use of an animal for experimental or other scientific purposes which may cause it pain, etc., while Article 3 of the Directive and Article 2 of the Convention apply to the use of animals for experiments for purposes including the manufacture of drugs, and other substances or products. Thus, *Directive 86/609/EEC* and *Convention ETS 123* apply unequivocally to all use of live animals in the production of mAbs, whether the antibodies are intended for use as research tools, for assays, or for therapeutic or diagnostic purposes.

Monoclonal Antibody Production

After an initial immunisation *in vivo*, immunocompetent cells are fused with myeloma cells *in vitro* to produce single hybridoma cells secreting the specific antibody. Consequently, all existing hybridoma cell lines are initially grown up in a stationary *in vitro* culture.

In the light of present knowledge it can be concluded that, for all levels of mAb production, one or more *in vitro* methods are scientifically acceptable and reasonably and practicably available. Such *in vitro* methods have the additional advantage of producing antibodies with very high immunoreactivities. A previous objection to the *in vitro* methodology was that significant practical effort was needed to concentrate spent culture fluid and produce useful

amounts of mAbs. However, modern technology provides a variety of economically acceptable *in vitro* systems which enable the generation of both high concentrations and/or high yields of mAbs. Thus, most production facilities and up-to-date research institutes are now producing all of their mAbs *in vitro*.

The use of the traditional method, which causes a considerable amount of pain and distress to the animals involved (3), is a matter of great concern. In this method, selected antibody-producing hybridoma cells are injected into the peritoneal cavity of compatible laboratory animals under aseptic conditions to produce rapidly progressive local tumours secreting mAbs in high titre in the ascitic fluid. Substantial pain and discomfort result from the following: a) the initial priming with the irritant pristane; b) the subsequent rapidly growing tumour (which may disseminate); c) the rate and volume of ascites production; and finally d) the procedures for, and frequency of, harvesting. Clearly, the use of this method in the majority of circumstances where it is not necessary and cannot be justified breaches the provisions of *Directive 86/609/EEC* and the *European Convention* and, as a consequence, such *in vivo* production should cease.

Where there is an exceptional need for an emergency therapeutic application, the *in vivo* production of mAbs should be allowed. In those cases where there is an existing regulatory approval for a therapeutic or diagnostic use, the ascites method can only be accepted until the end of the approval period. In addition, the ascites method should be allowed in other very exceptional circumstances, where verifiable efforts have failed to produce the mAb *in vitro*. In this situation, each animal experiment should be scientifically justified, and limited in terms of time and the number of animals to be used. Continuing efforts to produce the mAb *in vitro* would be expected. In themselves, convenience, "custom and practice", lack of equipment, and/or lack of familiarity with cell culture methods are not justifications for new or continued use of the ascites method.

Pristane continues to be used to encourage consistent ascitic, rather than solid, tumours. In such cases, it is usually satisfactory to give a single priming injection of 0.2ml pristane intraperitoneally 7-10 days before injecting 10^6 - 10^7 hybridoma cells. However, before resorting to the use of pristane, it must be borne in mind that this causes painful peritonitis (3-5) and other malignant effects (6, 7). The Dutch Code of Practice suggests that pristane should not be used (4), and Freund's complete and incomplete adjuvants have been suggested as possible alternatives (8, 9).

Animals should be inspected frequently by suitably trained personnel so that their clinical conditions can be assessed. Initially, the animals should be handled and inspected by such personnel twice a day and, if necessary, more frequently later on. Animals must be killed without delay when they show more than mild distress, overt tumour deposits or spread, or significant dehydration or cachexia.

The volume of ascites should not normally exceed 20% of the host body weight in mice and rats, in the absence of overt cachexia. A 20% increase in body weight is indicative of a very small, almost imperceptible, swelling of the abdomen. Ascites fluid has to be harvested on a single occasion only, either under terminal anaesthesia or *post mortem*.

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 Descriptors: polyclonal antibody, golf ball, titer quantity, comparison, Freund's, acylamide.
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Descriptors: one step footpad immunization, hybridoma, immunized mouse spleen, human respiratory virus, *helicobacter pylori*.
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Descriptors: abstract, methods, purification method, biotechnology industry, pharmaceuticals, mammalian cell culture, protein A, chromatography, pH, isoelectric point.
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 NAL call number: QP631 N37
 Descriptors: rabbits, isolation, purification, antibody formation, binding.
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 Descriptors: solid phase reactor, bismaleimide crosslinking reagent, goat, mouse, human, Fab fragments.
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Descriptors: disaccharide trehalose, mouse, monoclonal IgM antibodies, freeze-drying, storage, elevated temperatures, culture media, ascitic fluids.
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NAL call number: 448.3 Im62
Descriptors: human antibodies, fab fragments, antigen chase, chemical coordination, analytical method.
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NAL call number: QR1.I57
Descriptors: mice, vaccine delivery system, subcutaneously injection, IgG, antitoxin titers.
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Descriptors: high-cell-density culture, spin filter perfusion, bench-top bioreactor cell growth inhibition,, monoclonal antibody production.
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NAL call number: 381 J8224
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NAL call number: QR180 J6
Descriptors: high density culture of hybridoma, dialysis membrane, comparison with in vitro methods.
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NAL call number: QP601 M49
Descriptors: monoclonal antibody, epitope, serum, antigen binding, antibody combining site, ligand binding, bacteriophage, bacteriophage M13, antigen detection.
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Descriptors: cell culture, hen's egg, antibody production.

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NAL call number: QH585 A1I58
Descriptors: natural product synthesis, pharmaceutical synthesis, synthetic method.
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NAL call number: QR189 V32
Descriptors: mice, C57BL/J, BALB/cBy, tobacco mosaic virus, zona pellucida, ZP3 epitope, vaccine production.
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NAL call number: RB37 A1B56
Descriptors: alternative method, polyclonal antiserum baboon tissue rat arterial smooth muscle cells complementary DNA, gene expression.
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NAL call number: QH585.C97
Descriptors: hybridoma cell culture, interactive environmental factors.
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Descriptors: coat protein, virus antigen, virus antibody, bacteriophage, antigen recognition, cross reaction, mice.
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Descriptors: meeting abstract, tobacco, immunologic method, synthetic method.

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 Descriptors: mice, hybridoma cells, perfusion reactor, enzyme linked immunosorbent assay.
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 Descriptors: human, cloning, phage display technology, novel antibodies, recombinant molecules, diagnostic and therapeutic agents, patents, pharmacokinetics.
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 NAL call number: QR355.J6
 Descriptors: hepatitis B virus, synthetic peptide, epitope mapping, surface antigen, immune repertoire, macromolecules.
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 NAL call number: QR180 J6
 Descriptors: incomplete Freund's adjuvant, hybridoma cell inoculation, proteose peptone, thioglycollate, corn oil, mice, Inbred BALB/c.

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NAL call number: Z7994.L3A53
Descriptors: abstract, yeasts, insect cells, bacteria, transgenic animals, bioreactors.
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NAL call number: QR180 J6
Descriptors: immunization protocol, cell fusion protocol, cloning, animal model, myeloma cell.
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NAL call number: QR180 J6
Descriptors: therapeutic purposes, comparison with ascitic fluid, human immunoglobulin, isotype composition, synthetic method.
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NAL call number: QH324.9 S4B56
Descriptors: ascitic fluid, cell culture supernatant, isolation, cultured cells, hybridomas.
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NAL call number: TP248.13.A5
Descriptors: biotechnology methods, chromatography, culture media, hybridomas.
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NAL call number: QH301.F3
Descriptors: human, genetics, peptides, amino acids.
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NAL call number: QR180 J6
Descriptors: hybridoma cells, biosynthesis, flow cytometry, entrapment, detection, monoclonal antibody, isolation, purification.

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 Descriptors: Venezuelan equine encephalitis virus, arbovirus, inactivated virus vaccine, mice, drug delivery system, adjuvant, serum antibody, mucosal antibody, challenge protection.
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 Descriptors: biotechnology methods, pharmaceuticals, drug delivery systems, human disease treatment, therapeutic method, vaccines, liposome technology.
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 NAL call number: QL55 A1I43
 Descriptors: antigenic specificity, hybridoma, BALB/c mice, genetic engineering.
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 NAL call number: QR185.8.H9H9
 Descriptors: guinea pigs, delayed-type hypersensitivity, skin reaction, monoclonal antibodies, isolation and purification.
- Handa-Corrigan, A., S. Nikolay, et al. (1995). **Monoclonal antibody production in hollow fiber bioreactors: Process, control and validation strategies for manufacturing industry.** *Enzyme and Microbial Technology* 17(3):225-230, ISSN:0141-0229.
 NAL call number: TP248.E5E565
 Descriptors: hollow fiber bioreactors, commercial manufacturing, monoclonal antibodies, mouse hybridoma.
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 NAL call number: QL55 A1I43
 Descriptors: humoral and cellular immune response, adjuvants, injection route, species differences, rabbit, chicken, rodents, guinea pig, farm animals.
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 NAL call number: QH585.C97
 Descriptors: monoclonal biosynthesis, sedimentation based separator, cell flocculation.

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NAL call number: QH442.J69
Descriptors: mice, hybridoma, T0405 cells, IgG monoclonal antibody, hepatitis B, culture media.
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NAL call number: 389.8 J825
Descriptors: mouse, interleukin 1, concanavalin A, phytohemagglutinin, immune response, diet.
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Descriptors: mouse, anti-guinea pig monoclonal antibody, cell fusion.
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NAL call number: QH442.J69
Descriptors: hybridoma, cell culture media, microporous hollow fiber reactors, cytology.
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NAL call number: QH585.C97
Descriptors: small quantities, monoclonal antibodies, polypropylene hollow fiber membrane, cell density, cultured cells analysis, hamsters, hybridomas.
- Hein, M.B., Y. Tang, et al. (1991). **Evaluation of immunoglobulins from plant-cells.** *Biotechnology Progress* 7(5):455-461, ISSN:8756-7938.
NAL call number: TP248.2.B46.
Descriptors: *Saccharomyces cerevisiae*, tobacco plant, murine monoclonal antibody.
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NAL call number: Z7994.L3A5
Descriptors: monoclonal antibody production, laboratory animal, alternative method, in vitro.

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NAL call number: QR180 J6
Descriptors: alternative to ascites production in mice, cell culture, cell media, oxygen consumption, hybridomas.
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NAL call number: SF405.5 A3
Descriptors: mice, volume culture flasks, fermentors, in vivo versus in vitro methods.
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NAL call number: QR74.M65
Descriptors: alternatives to hybridoma technology, novel peptide, vaccines, fusion phage, monoclonal antibody, vaccine development.
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Descriptors: polyclonal antibodies, mammalian alternative, immunohistochemistry.
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Descriptors: IgY, cell associated antigens, CD14, p23, alternative to mammalian antibodies.
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Descriptors: lipopeptides, complete Freund's adjuvant, IgY, egg yolk.
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NAL call number: TP248.27 A53E97 1989
Descriptors: cell culture, biotechnology, cytology.

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NAL call number:QH573.T5
Descriptors: cochlear tissue, hybridoma supernatants, cell culture.
- Holzmann, D. (September 15, 1994). **Agracetus grows monoclonals in soybeans and corn plants.** *GEN: Genetic Engineering News* 14(1):34 ISSN:0270-6377.
NAL call number: QH442 G456
Descriptors: DNA, transgenic seeds, colon cancer, herpes.
- Hommel, U., I. Behn and S. Hauschildt (1996). **IgY antibodies as secondary reagent in FACS analysis.** *Alternativen zu Tierexperimenten: ALTEX* 13(Supplement 96):40-44, ISSN: 0946-7785.
Descriptors: alternatives to mammalian antibodies, avian vitellin antibodies, IgY, labeling, FAC analysis.
- Hsieh, C.S., S.E. Macatonia, A. O'Garra, and K.M. Murphy (February 1, 1995) **T cell genetic background determines default T helper phenotype development in vitro.** *Journal of Experimental Medicine* 181(2):713-721, ISSN:0022-1007.
NAL call number:448.8 J822
Descriptors: *Leishmania major*, T helper, in vitro, transgenic system, cultured cells, Inbred C57BL, mice.
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NAL call number: QR1.E9
Descriptors: whole cell immobilization, gel strength, cell leakage prevention.
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Descriptors: genetic engineering, immunoglobulin fragment, phage display, binding affinity, *Escherichia coli*, recombinant DNA technology.
- Jackson L.R., L.J. Trudel, J.G. Fox, and N.S. Lipman (1996). **Evaluation of hollow fiber bioreactors as an alternative to murine ascites production for small scale monoclonal antibody production.** *Journal of Immunological Methods* 189(2):217-231, ISSN: 0022-1759.
NAL call number: QR180 J6
Descriptors: bioreactor, hybridoma, hollow fiber membrane, ascites, enzyme linked immunosorbent assay, mouse.

- Jakobovis, A. (October 1995). **Production of fully human antibodies by transgenic mice.** *Current Opinion in Biotechnology* 6(5):561-566, ISSN:0958-1669.
NAL call number: TP248.13 C87
Descriptors: monoclonal antibodies, human immunoglobulin loci, antigens.
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NAL call number: QR180 J6
Descriptors: hybridomas, dialysis tubes, monoclonal antibody synthesis, tissue culture.
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NAL call number: TP248.E5E565
Descriptors: mice, immunoglobulin G, antibody yield, monoclonal antibody production, glutamine consumption rate.
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NAL call number: QP251 M64
Descriptors: mice, intrasplenic immunization, in-vitro fertilization, oocyte lysis, antigen localization, western blot.
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NAL call number: QH506.M45
Descriptors: monoclonal biosynthesis, isolation, purification, ascites, cell fusion, clone cells, hamsters, hybridomas, immunization, mice, Inbred BALB/c, rats, cultured tumor cells.
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NAL call number: 381 J8222
Descriptors: guinea pig adrenal glands, cell-surface antigens, membrane glycoproteins.
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NAL call number: QP601 A1N3
Descriptors: monoclonal antibody, hormones, cell immobilization, high cell density, growth rate, bioreactor, yield, productivity, metabolism, dissolved oxygen concentration.

- Kamihira, M., H. Yoshida, S. Iijima, and T. Kobayashi (1990). **Effects of oxygen aeration on production of monoclonal antibody in immobilized hybridoma cell bioreactor.** *Journal of Fermentation and Bioengineering* 69(5):311-312, ISSN:0922-338X.
NAL call number: QP601 A1J6
Descriptors: mouse, immobilization, alginates, polyurethane, cell culture, fluidized bed reactor, bioreactor, dilution rate.
- Karu, A.E., C.W. Bell, and T.E. Chin (1995). **Recombinant antibody technology.** *ILAR Journal* 37(3):132-141, ISSN:0018-9960.
NAL call number: QL55 A1I43
Descriptors: structure, function, gene expression, engineering, semi-synthetic.
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Descriptors: murine hybridoma, colon cancer, *Chlamyida trachomatis*, cell fusion, oxygen transfer, cell viability.
- Kim, H.W. and D.S. Suh (1995). **Study on the anti-hla antibody production using in vitro immunization technique.** *Korean Journal of Zoology* 38(2):186-195, ISSN:0440-2510.
Descriptors: human anti-HLA monoclonal antibody production, splenocytes, congenic mice, thymocyte culture, macrophage, lymphocyte, immunoglobulin, elisa.
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Descriptors: neutralizing antibody, anaphylatoxin, monoclonal antibody, synthetic peptide, epitope mapping, phage display inflammation.
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NAL call number: RC799 G37
Descriptors: gastrointestinal oncology, cancer therapy, cancer diagnosis, neoplastic cell lines, in vitro studies.

- Koval'chuk, N.V., L.N. Chernousova, and M.V. Raevskaia (July-August 1995). **Improving of methods of production and screening of monoclonal antibodies to Salmonella antigens.** [Optimizatsiia metodov polucheniia i skringa monoklonal'nykh antitel k sal'monelleznym antigenam.] *Klinicheskaya Laboratornaya Diagnostika* 4:39-41, ISSN:0869-2084.
 Descriptors: bacterial antigens, ascitic fluid, antigens, salmonella, monoclonal biosynthesis, Inbred BALB/c, mice.
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 NAL call number: QR189 V32
 Descriptors: mice, polymethyl methacrylate polystyrene, vaccine, antibody response.
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 NAL call number: QR189 V32
 Descriptors: antibody response, bovine serum albumin, influenza virus.
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 NAL call number: TP248.2.B46
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 NAL call number: Z7994.L3A5
 Descriptors: mass production, laboratory scale production, vivo and in vitro methods, German animal protection law, animal regulations, Switzerland, Netherlands, animal testing alternatives, animal welfare.
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 Descriptors: cell culture, regulations, alternative production methods.

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Descriptors: *Nicotiana tabacum*, plant growth, rate.
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NAL call number: QH442 A1G4
Descriptors: antibody fragments, filamentous bacteriophage, rabbit antibody, clones, rabbit spleen, bone marrow purified human platelet, plasminogen activator, binding affinity.
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Descriptors: genetic engineering, phage display, transgene, antibody structure.
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NAL call number: 47.8 AM33P
Descriptors: bird, poultry industry, immunoglobulin G.
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NAL call number: TP248.65 F66F66
Descriptors: polyclonal antibodies, human DNA dependent RNA polymerase II (HPP, II), chicken, immunoglobulin Y, gene isolation, transcription assay.
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NAL call number: TP248.3 B46
Descriptors: monoclonal biosynthesis, hybridomas, immunology, cultured cells, tissue culture.
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NAL call number: TP248.65.F66F66
Descriptors: cell culture, antibodies, biosynthesis, laboratory methods.

- Lerner, R.A., A.S. Kang, et al. (November 20, 1992) **Antibodies without immunization.** *Science* 258:1313-1314, ISSN: 0036-8075.
NAL call number: 470 SCI2
Descriptors: alternative method, *Escherichia coli*, combinatorial libraries.
- Linardos, T.I., N. Kalogerakis, L.A. Behie, and L.R. Lamontagne (1992). **Monoclonal antibody production in dialyzed continuous suspension culture.** *Biotechnology and Bioengineering* 39(5):504-510, ISSN:0006-3592.
NAL call number: 381 J8224
Descriptors: mouse hybridoma, Celligen bioreactor, cell growth, cell viability.
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Descriptors: in vitro immunization, somatic cell fusion, Epstein Barr virus transformation, human immunoglobulin gene libraries, phage display techniques.
- Liu, Y. and W. Xu (1995). **Comparative study on early and rapid method for production of rat monoclonal antibodies in vitro.** *Xi'an Yike Daxue Xuebao* 16(3):324-326, ISSN:0258-0659.
Descriptors: hemorrhagic fever, renal syndrome virus, immunoglobulin, hybridoma cell culture, synthetic methods.
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NAL call number: 472 N21
Descriptors: mice, hybridoma cell lines, human IgM, IgG, Ig kappa.
- Lösch, U. (1996). **How do the antibodies get into the chicken egg? [Wie kommen die antikörper ins hühnerei?] Alternativen zu Tierexperimenten: ALTEX** 13(Supplement 96):15-17, ISSN: 0946-7785.
Descriptors: chicken, IgY, IgG, IgA, IgY, yolk, yolk-sac, amnion, allantois.
- Lowrey, D.M., K. Meslovich, et al. (May 1994). **Monoclonal antibody production and purification using miniature hollow fiber cell culture technology.** *American Biotechnology Laboratory* 12(6):16-17, ISSN:0749-3223.
NAL call number: TP248.13.A5
Descriptors: mice, monoclonal biosynthesis, cell line, electrophoresis, polyacrylamide gel, hybridomas.

- Lundkvist, A. and B. Niklasson (1992). **Bank vole monoclonal antibodies against Puumala virus envelope glycoproteins: Identification of epitopes involved in neutralization.** *Archives of Virology* 126(1-4):93-105, ISSN:0304-8608.
NAL call number: 448.3 Ar23
Descriptors: antigen and epitope specificities, magnetic beads, hantavirus, fluorescent antibody technique.
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Descriptors: avidity, specificity, vasoactive intestinal polypeptide, rabbits, gastrin, glucagon, insulin, secretin.
- Ma, J.K.C. (1995). **Antibody expression in plants.** *ACS Symposium Series: Antibody Expression and Engineering* H.Y. Wang (ed.), 604:56-69, ISSN:0097-6156.
NAL call number: QD1.A45
Descriptors: bioreactors, large scale antibody production, antibody engineering.
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Descriptors: recombinant expression systems, cell culture techniques, protein assembly capabilities, bulk production, low cost.
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NAL call number: TA166.T72
Descriptors: plant hosts, inexpensive, plentiful antibody supply, literature review, transgenic plants, gene transfer.
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NAL call number: 442.8 B5236
Descriptors: coliphages genetics, genetic vectors, muramidase chemistry, bacteriophage M13, *Escherichia coli* enzymology.
- Mahana, W., and A. Paraf (1993). **Mice ascites as a source of polyclonal and monoclonal antibodies.** *Journal of Immunological Methods* 161(2):187-192, ISSN:0022-1759.
NAL call number: QR180 J6
Descriptors: BALB/c mice, pristane, antigen, egg ovalbumin, ascitic fluid, synthetic method.

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NAL call number: QH301 J68
Descriptors: in vitro cell culture, antibody titer, immunologic agent, pharmacokinetics, pharmaceutical synthesis, liquid chromatography, synthetic method.
- Margni, R.A., I.M. Borel, et al. (July 1992). **The proportion of symmetric and asymmetric IgG antibody molecules synthesized by a cellular clone (hybridoma) can be regulated by placental culture supernatants.** *Cellular Immunology* 142(2):287-295, ISSN:0008-8749.
NAL call number: QR180 C4
Descriptors: pregnancy, placenta, asymmetric IgG antibodies, antibody isolation and purification, Inbred BALB/c mice.
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NAL call number: 442.8 J8224
Descriptors: phage display technology, antibody fragments, alternative to rodents.
- Marks, J.D., A.D. Griffiths, et al. (July 1992). **By passing immunization: building high affinity human antibodies by chain shuffling.** *Biotechnology* 10: 779:783, ISSN:0733-222X.
Descriptors: bacteriophage, in vitro, genes, human antibodies.
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NAL call number: 381 J8224
Descriptors: death rate, insect cells, methods, device, stirred tank bioreactor, gas flow rate.
- Marx, P.A., R.W. Compans, et al. (1993). **Protection against vaginal SIV transmission with microencapsulated vaccine.** *Science* 260(5112):1323-1327, ISSN:0036-8075.
NAL call number: 470 Sci2
Descriptors: Simian immunodeficiency virus, macaque, human immunodeficiency virus, systemic priming, intramuscular plus oral route, biodegradable microspheres, drug delivery.

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NAL call number: Z7994.L3A5
Descriptors: ascites, pain, hybridoma technology, in vitro production, replacement, refinement, reduction, regulatory aspects.
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NAL call number: QH506.M45
Descriptors: monoclonal biosynthesis, ascites, cell culture instrumentation, hybridomas, mice, rats.
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NAL call number: QH585.C97
Descriptors: monoclonal antibody production, dialysis tubing comparison with ascites production, cost control.
- Matsumura, M. and F.R.P. Jr. Nayve (1995). **Effects of ammonium ion removal on growth and MAb production of hybridoma cells.** *Cytotechnology* 18(1-2):35-50, ISSN:0920-9069.
NAL call number: QH585.C97
Descriptors: mouse, hepatitis B surface antigen, perfusion culture, culture broth, cross flow filtration, membrane fouling, culture method, cryotechnology.
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NAL call number: QH613 H57
Descriptors: rats, hybridoma, mouse IgG1, brain cytology.
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NAL call number: RM300 I55
Descriptors: human lymphocyte, immunologic method.
- McGuill, M.W. and Rowan, A.N. (Winter 1989). **Refinement of monoclonal antibody production and animal well-being.** *ILAR News* 31(1):7-10, ISSN:0018-9960.
NAL call number: QL55.A1I43
Descriptors: mice, ascites tumors, pristane, distress.

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NAL call number: QH442.J69
Descriptors: flow cytometry, surface antibody fluorescence, hybridoma cell lines ATCC HB124, anti-idiotypic biosynthesis, culture media, hybridomas, mice.
- Meilhoc, E., K.D. Wittrup, and J.E. Bailey (1990). **Influence of dissolved oxygen concentration on growth, mitochondrial function and antibody production of hybridoma cells in batch culture.** *Bioprocess Engineering* 5(6):263-274, ISSN:0178-515X.
NAL call number: TP248.3.B563
Descriptors: dissolved oxygen, antibody, hybridoma, metabolism, cell proliferation, mixed tank reactor, cell culture.
- Mendez, M.J., H. Abderrahim, M. Noguchi, et al. (March 20, 1995). **Analysis of the structural integrity of YACS comprising human-immunoglobulin genes in yeast and in embryonic stem-cells.** *Genomics* 26(2):294-307, ISSN:0888-7543.
NAL call number: QH445.2 G45
Descriptors: mice, human monoclonal antibody production, cloning, mouse stem cells, cell lines.
- Mestecky, J. and J.H. Eldridge (1991). **Targeting and controlled release of antigens for the effective induction of secretory antibody responses.** *Current Opinion in Immunology* 3(4):492-495, ISSN:0952-7915.
NAL call number: QR180 C87
Descriptors: review, mouse, cellular immunity, vaccine, immunoglobulin A.
- Moro, A.M., M.T. Rodrigues, et al. (November 1994). **Multiparametric analyses of hybridoma growth on glass cylinders in a packed-bed bioreactor system with internal aeration. Serum-supplemented and serum-free media comparison for MAb production.** *Journal Immunological Methods* 176(1):67-77, ISSN:0022-1759.
NAL call number: QR180 J6
Descriptors: large scale production, bioreactor vessel, cost effective, culture media, hybridomas, affinity diffusion chambers.
- Murray, K., K. Gull, and A.J. Dickson (1996). **Dichloroacetate increases cell and antibody yields in batch cultures of a hybridoma cell lines.** *Biotechnology and Bioengineering* 49 (4):377-382, ISSN:0006-3592.
NAL call number: 381 J8224
Descriptors: pyruvate dehydrogenase, dichloroacetate, growth, metabolism, hybridoma cell line, laboratory scale, antibody yield.

- Naim, J.O. and C.J. Van Oss (1995). **Silicone gels as adjuvants: Effects on humoral and cell-mediated immune responses.** Zouhair Atassi, M. and G. S. Bixler, Jr., ed., In: *Advances in, Experimental Medicine and Biology, Vol. 383: Immunobiology of Proteins and Peptides VIII: Manipulation or Modulation of the Immune Response* Plenum Publishing Corp.: New York, NY, pp. 1-6, ISBN:0-306-45125-5.
 Descriptors: rat, mice, mammary implant, antibodies, arthritis, inflammatory disease, bones, joints, fasciae, connective and adipose tissue pathology.
- Nakamura, G.R., R. Byrn, et al. (November 1992). **Monoclonal antibodies to the extracellular domain of HIV-1IIIB gp160 that neutralize infectivity, block binding to CD4, and react with diverse isolates.** *AIDS Research and Human Retroviruses* 8(11):1875-1885, ISSN:0889-2229.
 Descriptors: hamsters, CD4 immunology, HIV envelope protein gp120, antibody specificity.
- Nakaoka, R., Y. Tabata, and Y. Ikada (1995). **Enhanced antibody production through sustained antigen release from, biodegradable granules.** *Journal of Controlled Release* 37(3):215-224, ISSN:0168-3659.
 NAL call number: RS201 C64J68
 Descriptors: mouse, intraperitoneal administration, ovalbumin, poly(DL-lactic acid) granules, IgG production, interleukin 1, in vitro.
- Nayve, F.R.Jr., T. Misato, M. Matsumura, and H. Kataoka (May 31, 1994). **HBs-MAb production in perfusion culture with selective ammonia removal system.** *Journal of Biotechnology* 34(3):217-225, ISSN:0168-1656.
 NAL call number: QH442.J69
 Descriptors: mice, serum-free perfusion cultures, hybridoma TO-405 cells, cell densities, perfusion cultures, cultured cells.
- Nilson, B., B. Akerstrom, and L. Logdberg (May 1987). **Cross reacting monoclonal anti-alpha 1-microglobulin antibodies produced by multi-species immunization and using protein G for the screening assay.** *Journal of Immunological Methods* 99(1):39-45, ISSN:0022-1759.
 NAL call number: QR180 J6
 Descriptors: BALB(c) mouse, hybridomas, alpha 1-microglobulin, monoclonal antibody biosynthesis.
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 NAL call number: 381 J8224
 Descriptors: filter perfusion system, animal cell culture, monoclonal antibody yield, proteins.

- Ohlin, M., H. Owman, M. Mach, and C.A. Borrebaeck (January 1996). **Light chain shuffling of a high affinity antibody results in a drift in epitope recognition.** *Molecular Immunology* 33(1):47-56, ISSN:0161-5890.
NAL call number: 448.3 Im62
Descriptors: human polyclonal and monoclonal antibodies, pathogens, toxins, hybridoma, coliphages, recombinant proteins.
- Omasa, T., M. Kobayashi, et al. (1995). **Enhancement of antibody production by growth factor addition in perfusion and hollow fiber culture systems.** *Biotechnology and Bioengineering* 48(6):673-680, ISSN:0006-3592.
NAL call number: 381 J8224
Descriptors: transferrin, bovine serum albumin (BSA), cell growth, hybridoma, perfusion culture.
- Overkamp, D., S. Mohammed-Ali, C. Cartledge, and J. Landon (1988). **Production of polyclonal antibodies in ascitic fluid of mice technique and applications.** *Journal of Immunoassay* 9(1):51-68, ISSN:0197-1522.
Descriptors: BALB/c mice, intraperitoneal versus subcutaneously immunization, insulin, antibody titers.
- Oyaas, K., T.E. Ellingsen, N. Dyrset, and D.W. Levine (1994). **Hyperosmotic hybridoma cell cultures: increased monoclonal antibody production with addition of glycine betaine.** *Biotechnology and Bioengineering* 44(8):991-998, ISSN:0006-3592.
NAL call number: 381 J8224
Descriptors: mouse, hybridoma cells, NaCl, sucrose, glycine betaine medium osmolality, physiology, titer, yield, osmoprotective compounds, stress.
- Ozturk, S.S. and B.O. Palsson (November-December 1991). **Growth, metabolic, and antibody production kinetics of hybridoma cell culture: 2. Effects of serum concentration, dissolved oxygen concentration, and medium pH in a batch reactor.** *Biotechnology Progress* 7(6):481-494, ISSN:8756-7938.
NAL call number: TP248.2.B46
Descriptors: murine hybridoma cell line, cell growth, viability, cell density, energy metabolism.
- Ozturk, S.S. and B.O. Palsson (1990). **Effects of dissolved oxygen on hybridoma cell growth, metabolism, and antibody production kinetics in continuous culture.** *Biotechnology Progress* 6(6):437-446, ISSN:8756-7938.
NAL call number: TP248.2.B46
Descriptors: viability, ATP, amino acid, cell proliferation, production, continuous stirred tank reactor, cell culture.

- Pakdel, F., F. Petit, et al. (1994). **Over expression of rainbow trout estrogen receptor domains in *Escherichia coli*: characterization and utilization in the production of antibodies for immunoblotting and immunocytochemistry.** *Molecular and Cellular Endocrinology* 104(1):81-93, ISSN:0303-7207.
NAL call number: QP187 A1M6
Descriptors: rabbits, rainbow trout, polyclonal antibodies, estrogen receptor, immunoblotting, antibody production.
- Pakkanen, R. and M. Neutra (1994). **Bovine colostrum ultrafiltrate: An effective supplement for the culture of mouse-mouse hybridoma cells.** *Journal of Immunological Methods* 169(1):63-71, ISSN:0022-1759.
NAL call number: QR180 J6
Descriptors: ultrafiltrate fraction, bovine colostrum, cell culture supplement, cultured mouse-mouse hybridomas, IgG, IgA antibodies.
- Pannell, R. and C. Milstein (1992). **An oscillating bubble chamber for laboratory scale production of monoclonal antibodies as an alternative to ascitic tumors.** *Journal of Immunological Methods* 146:43-48, ISSN:0022-1759.
NAL call number: QR180 J6
Descriptors: hybridomas, in vitro, contaminating proteins, monoclonal antibodies.
- Park, S.Y., and G.M. Lee (1995). **Enhancement of monoclonal antibody production by immobilized hybridoma cell culture with hyperosmolar medium.** *Biotechnology and Bioengineering* 48(6):699-705, ISSN:0006-3592.
NAL call number: 381 J8224
Descriptors: mice, hyperosmotic stress, S3H5-gamma-2bA2 hybridoma cells, hyperosmotic stress, product yield, antibody productivity.
- Park, S.H. and D.D.Y. Ryu (1994). **Cell cycle kinetics and monoclonal antibody productivity of hybridoma cells during perfusion culture.** *Biotechnology and Bioengineering* 44(3):361-367, ISSN:0006-3592.
NAL call number: 381 J8224
Descriptors: mouse, perfusion culture, hybridoma cell line, cell cycle phases, culture condition, production optimization, synthetic method.
- Perez, M.E., J. Gavilondo-Cowley, and M. Aguilar (1988). **Evaluation of different culture conditions for the in-vitro propagation of the hybridoma ifi-b6-c5 and the production of monoclonal antibodies.** *Interferon y Biotechnologia* 5(3):262-269, ISSN:0138-8878.
Descriptors: mouse, alternative, ascitic fluids, mass culture, bovine serum, human endothelial cell supernatant, roller cultures, cell density, immunoglobulin concentrations.

- Persson, B. and C. Emborg (1992). **A comparison of three different mammalian cell bioreactors for the production of monoclonal antibodies.** *Bioprocess Engineering* 8(3-4):157-163, ISSN:0178-515X.
NAL call number: TP248.3.B563
Descriptors: stirred tank reactors, mammalian cell culture, hybridoma cell growth, monoclonal antibody production.
- Peterson, N.C. (1996). **Recombinant antibodies: alternative strategies for developing and manipulating murine-derived monoclonal antibodies.** *Laboratory Animal Science* 46(1):8-14, ISSN:0023-6764.
NAL call number: 410.9 P94
Descriptors: protein interactions, therapeutic agents, monoclonal hybridoma production, computer assisted molecular modeling, in vitro techniques, phage display, reduce and replace animals.
- Petrossian, A. and G.P. Cortessis (April 1990). **Large-scale production of monoclonal antibodies in defined serum-free media in airlift bioreactors.** *Biotechniques* 8(4):414-422, ISSN:0736-6205.
NAL call number: RB37 A1B56
Descriptors: airlift bioreactors, hybridoma cell lines, serum free media, monoclonal isolation and purification.
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Descriptors: human Fab phage display library, peripheral blood lymphocytes, human immunodeficiency virus type-1, anti-Rev Fab clones.
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NAL call number: QR180 I425
Descriptors: IgY, hens, immunization, egg yolk.
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NAL call number: QR180 I425
Descriptors: IgY, hens, immunization, egg yolk.

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NAL call number: QL55 A1L33
Descriptors: female BALB/c, mice, ascites, production, hybridoma, pristane priming, adverse side-effects, necropsy.
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NAL call number: QR185.8.H9H9
Descriptors: human-mouse heterohybridomas, monoclonal antibody, IgG, interleukin 2, immunization, human cell.
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NAL call number: QH442.J69
Descriptors: cell culture, immobilized cell, optimization, glutamine, amino acid, fixed bed reactor, pore structure.
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NAL call number: TP248.24 B55
Descriptors: mouse, hybridoma cells, tubular reactor.
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NAL call number: QR180.3 D4
Descriptors: large scale production, stirred reactors, murine cell line.
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NAL call number: RB37 A1B56
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Descriptors: IgY, rheumatoid factors, comparison with mammalian antibodies.
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NAL call number: QH442.J69
Descriptors: chromatography, ion exchange, culture media, cytological techniques, monoclonal biosynthesis, cell count, hybridomas, mice.
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NAL call number: QR186.85 M6623 1995
Descriptors: human, immunoglobulin G, immunoglobulin, M, cell culture, contamination, cryopreservation, cloning techniques, screening.
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NAL call number: Z7994.L3A5
Descriptors: alternatives, distress, anxiety, suffering, antibody production.
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NAL call number: QH506.U34 v.84
Descriptors: non-toxic, non-allergenic, in vitro synthesis, human use.
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NAL call number: QH506.M45
Descriptors: mice, hybridomas, elisa, monoclonal antibody, anti-immunoglobulin, immunologic method, analytical method.

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NAL call number: IPS27338
Descriptors: cell culture, roller bottles, in vitro-production, monoclonal antibodies, laboratory scale, foetal, newborn, mouse, donor calf serum, culture, hybridoma, ascitic fluid.
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NAL call number: QD1 C4836 1994
Descriptors: DNA, genetics, corn, human diseases.
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Descriptors: hamsters, monoclonal biosynthesis, hybridomas, tissue culture methods.
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NAL call number: 448.39 So12
Descriptors: cell fusion, monoclonal antibodies, plant diseases, mango, *Xanthomonas campestris*, antibody specificity, epitopes, IgG, Inbred BALB/c mice.
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NAL call number: QH442 A1G4
Descriptors: mice, murine monoclonal antibodies, mutagenesis, nucleic acid, heteroduplexes, polymerase chain reaction.
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Descriptors: avian versus mammalian antibodies, radioimmunoassay, Freund's adjuvant.

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NAL call number: Z7994.L3A5
Descriptors: abstract, animal suffering, alternative method, reduction, refinement, replacement, IgY antibodies.
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Descriptors: Ig-Y technology, alternative method, chicken housing, immunization protocol.
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NAL call number: Z7994 L3A5
Descriptors: egg yolk, polyclonal antibodies, pain, immunocytochemistry.
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NAL call number: QR180.3 D4 v.86
Descriptors: pharmacological and toxicological evaluation, legal regulations, European Union, clinical trials.
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NAL call number: QH506 M45 no.45
Descriptors: spleen cells, myeloma cells, B lymphocytes, alternative to mouse immunization.
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NAL call number: 381 J8224
Descriptors: mouse human chimeric antibodies, SP2/0, mouse myeloma cells, conventional culture, hollow fiber system. bioreactor genetic engineering, immunoglobulin expression, growth medium, cones, fermentor.

- Schniering, A. R. Schade, and T. Hiepe (1996). **Development of an IgY based assay for the detection of *ascaris suum* antigens.** [Aufbau eines assays zur detektion von *Ascaris summ* antigenen auf der basis von IgY.] *Alternativen zu Tierexperimenten: ALTEX* 13(Supplement 96):5-9, ISSN: 0946-7785.
 Descriptors: copro antigen, IgY, enzyme immuno assay (EIA).
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 Descriptors: hybridoma cell lines , mouse myelomat cells, rat spleen cells. C3H mice.
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 Descriptors: chicken, adjuvants, lipopeptides, Freund's complete adjuvant, muramyl-dipeptide, myositis.
- Schmitt, J.J., U. Zimmermann, and G.A. Neil (February 1989). **Efficient generation of stable antibody forming hybridoma cells by electrofusion.** *Hybridoma* 8(1):107-115, ISSN:0272-457X.
 NAL call number: QR185.8.H9H9
 Descriptors: murine hybridomas, electrofusion protocols, temperature, bovine serum albumin.
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 NAL call number: QH585.C97
 Descriptors: monoclonal antibody biosynthesis, recombinant proteins, tissue culture methods, biotechnology methods.
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 Descriptors: IgY, White Leghorn, Freund's, egg yolk, precipitation.
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 NAL call number: QP601 A1J6
 Descriptors: cell culture, animal, hybridoma, cell proliferation, antibody, glucose, growth rate, optimization, mixed tank reactor, microfiltration, hollow fiber, perfusion.

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NAL call number: QH301.F3
Descriptors: human, amino acid, hydrogen bonding, peptide shape, carboxyl terminal, glycine, protein structure, systemic lupus erythematosus, recurrent thrombosis.
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NAL call number: TP248.13 A38
Descriptors: perfusion, instrumentation, cell division, cultured cells.
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Descriptors: hybridoma culture, pore size, serum concentration, antibody yield.
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NAL call number: QR180 C4
Descriptors: mice, Inbred CA, IgA, IgG binding factors, macrophages, T cells, immunoglobulin isotypes, biosynthesis, T-Lymphocytes, lymphokines.
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NAL call number: 381 J8222
Descriptors: ascitic fluid, Golgi apparatus, immunoenzyme techniques, hybridomas, mice, Inbred BALB/c, Inbred C57BL, rats, Sprague-Dawley.
- Spieker-Polet, H., P. Sethupathi, P.C. Yam, and K.L. Knight (September 26, 1995). **Rabbit monoclonal antibodies: generating a fusion partner to produce rabbit-rabbit hybridomas.** *Proceedings of the National Academy of Sciences* 92(20):9348-9352, ISSN:0027-8424.
NAL call number: 500 N21P
Descriptors: plasmacytoma cell lines, spleen cells, hybridomas, monoclonal biosynthesis, blood proteins, cell fusion.

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NAL call number: SF757.2 V38
Descriptors: bovine B lymphocytes, murine cell line, cell culture.
- Staak, C. (1996). **Egg yolk antibodies (IgY) in routine diagnostic work.** *Alternativen zu Tierexperimenten: ALTEX* 13(Supplement 96):73-75, ISSN: 0946-7785.
Descriptors: alternatives to mammalian antibodies, serological diagnosis, zoonoses, animal diseases.
- Steineker, F., J. Kreuter, and J. Loewer (1991). **High antibody titers in mice with polymethylmethacrylate nanoparticles as an adjuvant for HIV vaccines.** *Aids* 5(4):431-436, ISSN:0954-0121.
Descriptors: comparison, aluminum hydroxide formulations, human immunodeficiency virus.
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NAL call number: Z7994.L3A5
Descriptors: abstract, high titers, animal species, route of administration, antigen per dose, cytotoxicity.
- Stiftung Forshung 3R (September 1994). **MABs Without Mice?** *3R-INFO Bulletin* 2.
Descriptors: hollow fiber reactors, monoclonal antibody production, culture systems.
- Stills, H.F. (1994). **Polyclonal antibody production.** In: *The Biology of the Laboratory Rabbit* P.J. Manning, D.H. Ringler and C.E. Newcomber, eds., 2nd edition, Academic Press, Inc., New York, pp. 435-448, ISBN: 0-12-469235-4.
NAL call number: SF966.5 B56 1994
Descriptors: humoral antibody response, routes of injection, necrosis, inflammation, Freund's adjuvant, Squalene, TiterMax, Ribl, alum, lipopolysaccharides, liposomes, saponins.
- Stoll, T., C. Perregaux, U. Von Stockar, I.W. Marison (1995). **Production of immunoglobulin A in different reactor configurations.** *Cytotechnology* 17(1):53-63, ISSN:0920-9069.
NAL call number: QH585.C97
Descriptors: murine hybridoma line (Zac3), T-flask, stirred tank bioreactor, hollow fiber reactor, BALB/c mouse, ascites fluid.

- Stoltenborg, J.K., P.W. Tsao, et al. (September 1994). **A fluorescent cellular adhesion assay using insect cell produced human VCAM1.** *Journal of Immunological Methods* 175(1):59-68, ISSN:0022-1759.
NAL call number: QR180 J6
Descriptors: cell adhesion, cell line, flow cytometry, recombinant proteins, tumor cells, cultured.
- Takagi, M., K.I. Ohara, and T. Yoshida (1995). **Effect of hydrostatic pressure on hybridoma cell metabolism.** *Journal of Fermentation and Bioengineering* 80(6):619-621, ISSN:0922-338X.
NAL call number: QP601 A1J6
Descriptors: mice, cell growth, glucose consumption, Afp-27 cell line, synthetic method, immunological method.
- Tarzi, A. and F.B. Orlans (1995). **Directory of funding sources for scientific pursuit of alternatives.** In: *The World Congress on Alternatives and Animal Use in the Life Sciences: Education, Research, Testing, Alternative Methods in Toxicology and the Life Sciences, Volume 11*, Mary Ann Liebert, Inc. , New York, NY, pp.703-712, ISBN: 0913113662.
NAL call number: RA1199 A49 v.11 (in progress)
Descriptors: groups that fund alternatives research, some have funded in vitro antibody production methods, industrial, government, foundations, humane societies.
- Terashima, S., T. Ogawa, et al. (1993). **Immobilization of hybridoma cells with macroporous cellulosic support and improved production of monoclonal antibody.** *Seibutsu-Kogaku Kaishi* 71(3):165-170, ISSN:0919-3758.
NAL call number: QP601 A1N3
Descriptors: monoclonal antibody, polyethyleneimine, perfusion culture, fluidized bed bioreactor.
- Theodorou, N.A., P. Easterbrook, M. Tyhurst, and S.L. Howell (1981). **Islets of langerhans implanted in diffusion chambers do not initiate antibody production.** *Transplantation* 31(1):89-90, ISSN:0041-1337.
NAL call number: QP89 T73
Descriptors: rat, experimental surgery, homotransplantation, langerhansian islet, immunology, endocrine pancreas, endocrinology.
- Thompson, J., and T. Pope (February 16,1996). **Affinity maturation of a high-affinity human monoclonal antibody against the third hypervariable loop of human immunodeficiency virus: use of phage display to improve affinity and broaden strain reactivity.** *Journal of Molecular Biology* 256(1):77-88, ISSN:0022-2836.
NAL call number: 442.8 J8224
Descriptors: therapeutic monoclonal antibody, in vitro, HIV antibodies, peptide fragments, affinity genetics, cloning.

- Titova, N.G., I.V. Razina, et al. (November-December 1995). **The production of hybridomas producing monoclonal antibodies to the causative agent of melioidosis by using antigen-stimulated lymphocytes in an in vitro system.** [Poluchenie gibridom-produtsentov monoklonal'nykh antitel k vozбудitel'iu melioidoza s ispol'zovaniem limfotsitov stimulirovannykh antigenom v sisteme in vitro.] *Zhurnal Mikrobiologii Epidemiologii I Immunobiologii* (6):82-83, ISSN:0372-9311.
NAL call number: 448.3 Z4
Descriptors: biosynthesis, monoclonal antibodies, bacterial immunology, *Burkholderia pseudomallei*, hybridomas immunology, mice, Inbred BALB/c.
- Tsurushita, N., H. Fu, and C. Warren (June 12, 1996). **Phage display vectors for in vivo recombination of immunoglobulin heavy and light chain genes to make large combinatorial libraries.** *Gene* 172(1):59-63, ISSN:0378-1119.
NAL call number: QH442 A1G4
Descriptors: bacteriophage, genetic vectors, immunoglobulins, recombinant genetics, nucleotidyltransferases metabolism.
- Tu, L., D. Huang, et al. (1994). **Regulatory action of Astragalus saponins and Buzhong Yiqi compound on synthesis of nicotinic acetylcholine receptor antibody in vitro from myasthenia gravis.** *Chinese Medical Journal* 107(4):300-303, ISSN:0366-6999.
Descriptors: saponin, Chinese herb, immunomodulation, Chinese medicine, human cell culture.
- Valdes, R., T. Diaz, et al. (1995). **Sendai virus removal and inactivation during monoclonal antibody purification.** *Biotechnologia Aplicada* 12(2):115-119.
Descriptors: viral contamination, risk, human, ascitic fluid, antibody purification method.
- Van Der Kamp, M. and W. de Leeuw (October 1996). **Short review of in vitro methods for monoclonal antibodies.** *NCA Newsletter* 3:10-12.
Descriptors: Netherlands Centre Alternatives to Animal Use (NCA), spinner flasks, dialysis membrane, roller bottles, hollow fiber, fermentors.
- Van Der Pol, J.J., B. Joks, et al. (1995). **On-line control of an immobilized hybridoma culture with multi-channel flow injection analysis.** *Journal of Biotechnology* 43(3):229-242, ISSN:0168-1656.
NAL call number: QH442.J69
Descriptors: glucose, glutamine concentrations, flow injection analysis, specific monoclonal antibody production, lactate, ammonium concentrations, amino acid concentrations.

- Van Der Pol, L., G. Zijlstra, M. Thalen, and J. Tramper (1990). **Effect of serum concentration of production of monoclonal antibodies and on shear sensitivity of a hybridoma.** *Bioprocess Engineering* 5(6):241-245, ISSN:0178-515X.
NAL call number: TP248.3.B563
Descriptors: concentration, monoclonal antibody, environmental factor, hybridoma, production, bioreactor, continuous stirred tank reactor, cell culture, continuous process, culture medium, animal serum.
- Van Wyngaardt, W., D.H. Du Plessis, S. Van Wyngaardt, J.A. Verschoor (June 1992). **Production and properties of monoclonal antibodies against African horse sickness virus, serotype 3. Onderstepoort.** *Journal of Veterinary Research* 59(2):129-133, ISSN:0030-2465.
Descriptors: guinea pigs, Inbred BALB/c mice, C57BL polyethylene glycol mediated cell fusions.
- Van Der Pol, L., G. Zijlstra, et al. (1990). **Effect of serum concentration on production of monoclonal antibodies and on shear sensitivity of a hybridoma.** *Bioprocess Engineering* 5(6):241-246, ISSN:0178-515X.
Descriptors: rat, mouse, cell death rate, serum content, hybridomas, stirred tank reactor.
- Vaughan, T.J., A.J. Williams, et al. (1996). **Human antibodies with sub-nanomolar affinities isolated from a large non-immunized phage display library.** *Nature Biotechnology* 14(3):309-314, ISSN:1087-0156.
NAL call number: QH442 B5
Descriptors: alternative to hybridoma technology, gene segments, library format, hormones, pharmaceuticals, hybridoma technology, high affinity monoclonal antibodies, genetic engineering, purification method.
- Wahl, M.F., G. An, and J.M. Lee (May 1995). **Effects of dimethyl sulfoxide on heavy chain monoclonal antibody production from plant cell culture.** *Biotechnology Letters* 17(5):463-468, ISSN:0141-5492.
NAL call number: QR53.B56
Descriptors: mice, dimethyl sulfoxide, *Nicotiana tabacum*, cell suspensions, cell growth, monoclonal antibodies, cell lines.
- Wang, G.Z., W.Y. Zhang, D. Freedman, and L. Eppstein (1992). **Use of packed bed reactor for production of monoclonal antibodies.** *Molecular Biology of the Cell* 3(Supplement):185A, ISSN:1059-1524.
NAL call number: QH604 C452
Descriptors: abstract, animal hybridoma cells, gamma interferon, immunologic method.

- Ward, R.L., M.A. Clark, J. Lees, and N.J. Hawkins (January 16, 1996). **Retrieval of human antibodies from phage-display libraries using enzymatic cleavage.** *Journal of Immunological Methods* 189(1):73-82, ISSN:0022-1759.
NAL call number: QR180 J6
Descriptors: human IgG1, kappa gene, pericolic lymph node, bacteriophage M13, recombinant proteins.
- WARDS (Summer 1990). **Monoclonal antibody production.** *Science and Animal Care* 1(1):1,4
NAL call number: HV4701 S35
Descriptors: animal care, cell culture, monoclonal, ascites, roller culture.
- Warren, H.S. and L.A. Chedid (1988). **Future prospects for vaccine adjuvants.** *CRC Critical Reviews in Immunology* 8(2):83-101, ISSN:0917-3355.
NAL call number: QR180 C78
Descriptors: aluminum compounds, polyanions, hydrophobic compounds, surfactants, Freund's incomplete adjuvant, liposomes, saponin.
- Weichert, H., Falkenberg, F.W., et al. (1995). **In vitro production of monoclonal antibodies in high concentration in a new and easy to handle modular minifermenter.** *In: Alternative Methods in Toxicology and the Life Sciences Series, Vol. 11, Proceedings of the World Congress on Alternatives and Animal Use in the Life Sciences: Education, Research, Testing* A.M. Goldberg and L.F. M. van Zutphen, eds., Mary Ann Liebert, Inc.: New York, NY., pp. 253-259, ISBN:0-913-113-66-2.
NAL call number: RA1199 A49
Descriptors: high density culture of hybridoma, dialysis membrane, comparison with in vitro methods.
- Weichert, H., M. Krane, et al. (1994). **In vitro production of monoclonal antibodies in high concentration in a new and easy to handle modular minifermenter.** *In Vitro Toxicology* 7(2):193, ISSN:0888-319X.
NAL call number: RM300 I55
Descriptors: meeting abstract, mouse, ascitic fluid production, hybridoma, high density cell culture, synthetic method, immunologic method.
- Whitelam, G.C., W. Cockburn, and M.R. Owen (November 1994). **Antibody production in transgenic plants.** *Biochemical Society Transactions* 22(4):940-944, ISSN:0300-5127.
NAL call number: QD415 A1B58
Descriptors: monoclonal antibody biosynthesis, transgenic biotechnology, gene expression, immunoglobulins, tobacco plant.

- Wolff, K.L., B.W. Hudson, R.R. Ormsbee, and M.G. Peacock (October 1976). **Production of antibody in induced granulomas.** *Journal of Clinical Microbiology* 4(4):384-387, ISSN:0095-1137.
NAL call number: QR46 J6
Descriptors: granulomas, rabbit, plastic golf balls, cellular migration, titers.
- Wongsamuth, R. and P.M. Doran (1996). **Production of monoclonal antibody by tobacco hairy roots.** *211th American Chemical Society National Meeting, New Orleans, Louisiana, USA, March 24-28, 1996. Abstracts of Papers, American Chemical Society* 211 (1-2): BIOT 166, ISSN:0065-7727.
Descriptors: genetic engineering, biotechnology, production levels, bioreactor, clones, transformation, product, yield.
- Xie, L. and D.I. Wang (1994). **Applications of improved stoichiometric model in medium design and fed-batch cultivation of animal cells in bioreactor.** *Cytotechnology* 15(1-3):17-29, ISSN:0920-9069.
NAL call number: QH585.C97
Descriptors: nutrient concentrations, toxic byproducts, ammonia, lactate, tissue culture methods, cell line, hybridomas, mice.
- Yang, Y.J. and R.F. Coico (February 1996). **In vitro enhancement of antibody responses by T delta cells expressing, IgD receptors.** *Cellular Immunology* 168(1):107-116, ISSN:0008-8749.
NAL call number: QR180 C4
Descriptors: murine T cells, adjuvants, antibody producing cells, IgD, T-Lymphocytes, cultured cells.
- Yoon, S.J. and K.B. Konstantinov (1994). **Continuous, real-time monitoring of the oxygen uptake rate (OUR) in animal cell bioreactors.** *Biotechnology and Bioengineering* 44(8):983-990, ISSN:0006-3592.
NAL call number: 381 J8224
Descriptors: murine myeloma cell line, monoclonal antibodies, laboratory scale cultivation, industrial scale perfusion culture.
- Zamboni, A., I. Giuntini, et al. (1994). **Production of mouse monoclonal antibodies using a continuous cell culture fermenter and protein G affinity chromatography.** *Cytotechnology* 16(2): 79-87, ISSN:0920-9069.
NAL call number: QH585.C97
Descriptors: mice, anti-alpha fetoprotein, monoclonal antibodies, double membrane stirrer, electrophoresis, chromatography, hybridoma technology.

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NAL call number: 381 J8224
Descriptors: continuous cultures, kinetic model, productivity, nutrients, toxic products, mathematical simulation.
- Zeng, Q.S., H. Takeyama, S. Kanda, and R.F. Irie (1994). **Serum preparation and methods for the large-scale production of IgG monoclonal antibody.** *Human Antibodies and Hybridomas* (1-2):75-80, ISSN:0956-960X.
Descriptors: fetal calf serum, serum free culture media, mouse myeloma, cell line, cattle, mice, hybridomas.
- Zhao, X.(1995). **Use of electric field mediated cell fusion to produce hybridomas secreting monoclonal antibodies.** *Methods in Molecular Biology* 45:61-67, ISSN:1064-3745.
NAL call number: QH506.M45
Descriptors: cell fusion, hybridomas, cytology, immunologic techniques, culture media, medical instrumentation, mice, Inbred BALB/c.
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NAL call number: SF757.2 V38
Descriptors: Freund's adjuvant, TiterMax, pigs, goats, mice, sequential immunization procedures, viral diseases, immune response, swine diseases.
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Descriptors: hybridoma, BALB/c mice, recombinant CD4 molecules, epitope recognition, competitive binding test, ascitic fluid.

Guidelines and Policies

Amyx, H.L. (1987). **Control of animal pain and distress in antibody production and infectious disease studies.** *Journal of the American Veterinary Medical Association* 191:1287-1289, ISSN:0003-1488.

NAL call number: 41.8 AM3

Descriptors: non-inflammatory alternatives, Freund, ribi, injection site, footpads, dosage, restraint.

Balk, R. (1990). **Ethical issues of adjuvant use.** In: *Adjuvants: Advances in Technology and Alternatives to Freund's* (Conference Proceedings). The Animal Resources Centre and the Faculty of Graduate Studies and Research, McGill University: Montreal, Quebec, pp. 15-18.

Descriptors: science, ethics, suffering.

Canadian Council on Animal Care (1993). **Categories of invasiveness in animal experiments.** In: *Guide to the Care and Use of Experimental Animals* E.D. Olfert, B.M. Cross, and A.A. McWilliam (eds.), 2nd edition, Vol. I, pp.201-202, ISBN:0-919-08718-3.

NAL call number: SF406 C36

Descriptors: distress, pain, discomfort.

Canadian Council on Animal Care (1993). **CCAC guidelines on acceptable immunological procedures.** In: *Guide to the Care and Use of Experimental Animals* E.D. Olfert, B.M. Cross, and A.A. McWilliam (eds.), 2nd edition, Vol. I, pp.203, ISBN:0-919-08718-3.

NAL call number: SF406 G85 1993

Descriptors: routes of administration, dosage, induction of ascites fluid, injection sites.

de Leeuw, W.A. and P. de Greeve (October 1996). **Production of polyclonal and monoclonal antibodies in the Netherlands.** In: *Second World Congress on Alternatives and Animal Use in the Life Sciences October 20-24, 1996, Utrecht, The Netherlands*, J.B.F. van der Valk and L.F.M. van Zutphen, eds., *Alternatives to Laboratory Animals: ATLA* 24(Special Issue):182, ISSN:0261-1929.

NAL call number: Z7994.L3A5

Descriptors: abstract, codes of practice, scientific societies, reduction, mice.

Grumstrup-Scott, J. and D.D. Greenhouse (Spring 1988). **NIH intramural recommendations for the research use of complete Freund's adjuvant.** *ILAR News* 30(2):9 ISSN:0018-9960.

NAL call number: QL55.A1I43

Descriptors: immunization, footpad, peritoneal exudate, judicious use, inflammatory response.

- Hanly, W.C. (January 1995). **UIC guidelines for the use of adjuvants in animals.** *BRL Bulletin* (10)1:1-4.
NAL call number: IP5G6428
Descriptors: Freund's, laboratory hazard, dose, emulsion, injection site.
- Hendriksen, C. and E. Claassen (October 1996). **Polyclonal and monoclonal antibodies: Guidelines for their production.** In: *Second World Congress on Alternatives and Animal Use in the Life Sciences October 20-24, 1996, Utrecht, The Netherlands*, J.B.F. van der Valk and L.F.M. van Zutphen, eds., *Alternatives to Laboratory Animals: ATLA* 24(Special Issue):181, ISSN:0261-1929.
NAL call number: Z7994.L3A5
Descriptors: abstract, Freund's Complete Adjuvant, adverse effects, welfare, immunization protocols, chicken egg-yolk antibodies, ascites.
- Hendriksen, C., J. Rozing, M. Van Der Kamp, and W. De Leeuw (1996). **The production of monoclonal antibodies: Are animals still needed?** *Alternative to Laboratory Animals: ATLA* 24(1):109-110, ISSN:0261-1929.
NAL call number: Z7994 L3A5
Descriptors: monoclonal antibody, production, laboratory animal, alternative method, in vitro.
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NAL call number: QL55 A1I43
Descriptors: welfare, recommendations, selection of animals, priming agents, Freund's adjuvant, alternatives, injection sites.
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NAL call number: Z7994.L3A5
Descriptors: mass production, laboratory scale production, vivo and in vitro methods, animal regulations, German animal protection law, Switzerland, Netherlands, animal testing alternatives, animal welfare.
- Marx, U., M.J. Embleton, R. Fischer, F.P. Gruber, et al. (1997). **Monoclonal antibody production: The report and recommendations of ECVAM Workshop 23.** *Alternatives to Laboratory Animals: ATLA* 25:121-137, ISSN:0261-1929.
NAL call number: Z7994.L3A5
Descriptors: ascites, pain, hybridoma technology, in vitro production, replacement, refinement, reduction, regulatory aspects.

- McGuill, M.W. and Rowan, A.N. (Winter 1989). **Refinement of monoclonal antibody production and animal well-being.** *ILAR News* 31(1):7-10, ISSN:0018-9960.
NAL call number: QL55.A1I43
Descriptors: mice, ascites tumors, pristane, distress.
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Descriptors: immunization, footpad, peritoneal exudate.
- Rowan, A.N. (1995). **The third R: Refinement.** *Alternative to Laboratory Animals: ATLA* 23:332-346, ISSN:0261-1929.
NAL call number: Z7994 L3A5
Descriptors: alternatives, distress, anxiety, suffering, antibody production.
- Straughan, D.W. (October 1996). **Prospects for European guidelines for polyclonal and monoclonal antibody production.** In: *Second World Congress on Alternatives and Animal Use in the Life Sciences October 20-24, 1996, Utrecht, The Netherlands*, J.B.F. van der Valk and L.F.M. van Zutphen, eds., *Alternatives to Laboratory Animals: ATLA* 24(Special Issue):183, ISSN:0261-1929.
NAL call number: Z7994.L3A5
Descriptors: abstract, alternatives, best practice, bleeding, immunization, ascites, humane endpoints.
- Toth, L.A., A.W. Dunlap, G.A. Olson, and J.R. Hessler (March 1989). **An evaluation of distress following intraperitoneal immunization with Freund's adjuvant in mice.** *Laboratory Animal Science* 39(2):122-126, ISSN:0023-6764.
NAL call number: 410.9 P94
Descriptors: mice, intraperitoneal, food intake, body weight, neutrophilia, granulomatous peritonitis.

Books and Proceedings

- Birch, J.R. and E.S. Lennox (1995) *Monoclonal Antibodies: Principles and Applications* Wiley-Liss: New York, 344 p., ISBN:0-471-05147-0.
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- Borrebaeck, C.A. (1995) *Antibody Engineering* Oxford University Press: New York, Oxford, 2nd ed., 390 p., ISBN:0-19-509150-7.
NAL call number: TP248.65 I49A57 1995
- Cambrosio, A. and P. Keating (1995) *Exquisite Specificity: The Monoclonal Antibody Revolution* Oxford University Press: New York 272p., ISBN:0-19-509741-6.
NAL call number: QR186.85 C35 1995
- Capra, J. D. (1996) *Antibody Engineering* Chemical Immunology Series, Vol. 65, Karger: Basel, Switzerland; New York, NY, 200p., ISBN:3-8055-6356-6.
- Cunningham, C. (1995) *Approaches in Developing Human Antibodies and Antibody Fragments* R G Landes, 157p., ISBN:1-57059-272-1.
- Davis, W.C. (1995) *Monoclonal Antibody Protocols* Humana Press: Totowa, N.J., 264p., ISBN:0-89603-308-2.
NAL call number: QH506 M45 no.45
- Delves, P. (1995) *Antibodies Applications: Essential Techniques* Wiley Oxford: Chichester, NY, 151p., ISBN:0-471-95698-8.
- Fanger, Michael W (1995) *Bispecific Antibodies* R.G. Landes: Austin, TX, 151p., ISBN:1-57059-166-0.
NAL call number: QR186.7 B57 1995
- Goding, J.W. (1996) *Monoclonal Antibodies: Principles and Practice* 3rd ed, Academic Press Inc.: London, San Diego, 512p., ISBN:0-12-287023-9.
NAL call number: IPM960628706
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NAL call number: QH301 N32 v.282

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- Liddell, J.E. and I. Weeks (1995) *Antibody Technology: A Comprehensive Overview* Bios Scientific: Oxford, 146 p., ISBN: 1-872748-87-2.
NAL call number: QR186.7 L54 1995
- Malik, V. S. and E.P. Lillehoj (1994) *Antibody Techniques: A Guide for Nonimmunologists* Academic Press: London, San Diego, CA, 353p., ISBN:0-12-466460-1.
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NAL call number: QR186.85 M38 1993
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NAL call number: QH506 M45 no.51
- Powell, M.F. and M.J. Newman (1995) *Vaccine Design: The Subunit and Adjuvant Approach* Pharmaceutical Biotechnology Vol. 6, Plenum Press, NY, 949p., ISBN:0-306-44867-X.
NAL call number: RS380 P53 v.6
- Ritter, M.A. and H.M. Ladyman (1995) *Monoclonal Antibodies: Production, Engineering and Clinical Application*, Postgraduate Medical Science Series Vol. No. 3, Cambridge University Press: New York, NY, 500p., ISBN:0-521-47354-3.
NAL call number: QR186.85 M6623 1995
- Rotheim (1995) *Bioengineered Protein Drugs: Antibodies, Blood Proteins* Business Communications Co.: Norwalk, CT, 282p., ISBN:0-614-10922-1.
- Stewart-Tull, D. E. (1995) *The Theory and Practical Application of Adjuvants*, J. Wiley: Chichester, England; New York, NY, 250p., ISBN:0-471-95170-6.

- Terhorst, C. P. , D. Malavasi, and A. Albertini, A. (1993) *Generation of Antibodies by Cell and Gene Immobilization* The Year in Immunology Ser. Vol.. 7, Karger: Basel, New York, 246p., ISBN: 3-8055-5714-0.
NAL call number: QR186.85 B56 1992
- Thibeault, C.A. and L.M. Savage (1996) *Antibody Engineering: New Technologies, Applications and Commercialization* IBC Biomedical Library Series, Southbridge, MA, 350p., ISBN:1-57936-001-7.
- Wang, H.Y. and T. Imanaka (1995) *Antibody Expression and Engineering* ACS Symposium Series Vol. 604, American Chemical Society: Washington, DC, 154p., ISBN:0-8412-3314-4.
NAL call number: QD1 A45 no.604
- Zanetti, M.(1995) *The Antibodies* Harwood Academic Publishers: Basel, Switzerland, 200p., ISBN:3-7186-0610-0.
- Zola, H.(1994) *Monoclonal Antibodies: The Second Generation* Bios Scientific Publishers: Oxford, United Kingdom, Herndon, VA. 250p., ISBN:1-872748-78-3.
NAL call number: QR186.85 M6624 1995

A Selected List of Company and Institute Resources Providing New Technologies

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Antibodies Inc.

P.O. Box 1560, Davis, CA 95617, USA

Telephone: (916) 758-4400, Fax: (916) 758-6307

E-mail: antiinc@aol.com

Manufacture monoclonal and polyclonal antibodies, custom antiserums, and diagnostic kits. Production via cell culture, fermentors, hybridomas, and small reactors.

BIOCULT

Antibody Production Centre

Academic Business Centre of Leiden University

Niels Bohrweg 11-13 K2, 2333 CA, Leiden, The Netherlands

Telephone: (31)-71-21 54 43 or (31)-71-27 68 62, Fax: (31)-71-21 72 72 (from U.S. 31-715217272)

E-mail: appeldoo@chem.leidenuniv.nl

Independent contract house for cell derived monoclonal antibodies. Activities focus on in vitro cultures. Use hollow fiber bioreactor perfusion systems and have processed over 25 different cell lines.

Biodesign International

105 York Street

Kennebunk, ME 04043, USA

Telephone: (207) 985-1944, Fax: (207) 985-6322

E-mail: info@biodesign.com

URL: <http://www.biddeford.com/~biodesin/bio/custom2.html>

Offers custom monoclonal antibody development for research and manufacturing. In vitro monoclonal antibody production services utilizing bioreactors and serum-free medium. Ascites production in mice.

BioInvent Production AB

S-223 70 Lund, Sweden

Telephone: +46-46-168550, Fax: +46-46-2110806

E-mail: info@bioinvent.se

<http://www.bioinvent.se>

Swedish company specializing in the development and production of monoclonal antibodies, including antibody engineering, cellular technology, cell culture and purification. Offering a choice of technologies available for continuous perfusion culture (Cellex, Acusyst, Xcell, and Opticell systems).

BIOMEDA

P.O. Box 8045, Foster City, CA 94404, USA

Telephone: (415) 341-8787, Toll free: 1- (800) 341-8787 or , Fax: (415) 341-2299

email: support@biomeda.com

URL: <http://www.biomeda.com>

Offer a wide variety of monoclonal and polyclonal antibodies for research purposes produced in mice, rabbits, and goats. In vitro and cell culture technologies used in production when possible.

Biosynthesis Incorporated

Post Office Box 28

Lewisville, TX 75067-0028, USA

Telephone: (972) 420-8505, Toll free: (800) 227-0627, Fax: (972) 420-0442

E-mail: biosyn@onramp.net

URL: <http://www.biosyn.com>

Offer chicken polyclonal antibody services. Option to store eggs for an extra charge. IgY extraction from one dozen eggs. Polyclonals also available from rabbits, sheep and goats. Monoclonals produced by ascites in mice.

Cellco, Inc. (see Spectrum)

12321 Middlebrook Road, Suite 210, Germantown, MD 20874, USA

Telephone: (301) 916-1000, Toll free: (800) 253-1803, Fax: (301) 916-1010

email: cellco@cellco.com

URL: <http://www.spectrumlabs.com>

Producers of CELLMAX®, artificial capillary cell culture systems designed to culture hybridoma, lymphocytes, endothelial cells and other cell types.

The Centre for Biomedical Technology (BMTC)

Faculty of Medical Sciences

University of Groningen

P.O. Box 30.001

9700 RB Groningen, The Netherlands

Telephone +31-50 3614776 or +31 50 3615018, Fax: +31-50 3633113

E-mail: r.m.j.hoedemakers@med.rug.nl

URL: <http://www.med.rug.nl/bmtc/medbiot/ivdlab/ivdlab.htm>

Developed BIAcore an analytical biosensor for measuring interactions between molecules in real time. BIAcore is capable of the following applications: measurement of concentrations in serum and supernatant, affinity constants of antigen-antibody interactions, screening hybridomas for IVD test kits, DNA-protein interactions, functionality of modified proteins or antibodies, optimizing conditions, for column chromatography, screening fractions of protein purifications. Service centre for in vitro diagnostics offers the facility and expertise for production of recombinant proteins, PCR development and PCR-ELISA detection, production of monoclonal and polyclonal antibodies.

CLB Biotechnology Services

Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands

Telephone: +31-(0)20-512-3549, Fax: +31-(0)20-512-3550 (from U.S. 31-205123550)

Specialize in contract production of monoclonal antibodies intended for *in-vivo* application and biosafety testing of biotechnological products. Antibodies produced using in-vitro methods.

CytRx Corp.

154 Technology Pkwy., Norcross, GA 30092, USA

Telephone: (770) 368-9500, Titermax Telephone: (800)-345-2987, Fax: (770) 447-8875

email: titermax@cytrx.com

URL: <http://www.titermax.com>

Produces of Titermax®, a synthetic alternative to Freund's adjuvant for monoclonal and polyclonal antibody production.

Cytogen Corp.

600 College Rd., E., Princeton, NJ 08540-5308, USA

Telephone: (609) 987-8200, Fax: (609) 452-2975

E-mail: cytoinfo@corpcomm.cytogen.com,

URL for catalog and newsletter: <http://www.cytogen.com>

Research and development of diagnostic and therapeutic substances such as monoclonal antibodies for cancer patients. Antibodies produced using hollow fiber bioreactors. Antibodies purified to a high purity suitable for in vivo use. Contract manufacturing of antibodies offered.

DiagXotics

27 Cannon Road, Wilton, CT 06897, USA

Telephone: (203) 762-0279, Toll free: (800) 676-2927, Fax: (203) 762-2378

email: john@csni.org

Manufacturers of a simple "No-Mouse" system in which a gas permeable bag is used to grow hybridoma cells for monoclonal antibody production. No special machinery required other than a tissue culture hood, 37°C incubator, and a low speed centrifuge.

Eramus Centre for Animal Research (EDC)

Erasmus University (FGG)

P.O. Box 1738, 3000 DR, Rotterdam, The Netherlands

Telephone: +31-(0)10-4087587, Fax: +31-(0)10-4361425 (from U.S. 31-104361425)

E-mail: buro@edc.fgg.eur.nl

URL: <http://www.fgg.eur.nl/FGG/EDC/>

Produce monoclonal antibodies in an artificial culture system (Integra Biosciences Technomouse) upon request and for experimental use only. Most monoclonal antibodies produced in protein free medium without the use of serum.

Facilitair Hybridoma Laboratorium

Institute for Infectious Diseases and Immunology

Faculty of Veterinary Medicine

Utrecht University

Yalelaan 1, 3584 CL Utrecht, The Netherlands

Telephone: +31 30 2531872 or /4358, Fax: +31 30 2533555 (from U.S. 31-302533555)

e-mail: p.kooten@vetmic.dgk.ruu.nl or w.eden@vetmic.dgk.ruu.nl

Producers of MoAbs in protein free medium in bulk culture and concentrated by ultra-filtration (30 kD filter). Product has a purity of +/- 80%. Higher percentage purity is available on request.

Fuzhou Maxim Biotech, Inc.

566 Gunyeh Rd.

Fuzhou, Fujian 350002, PR China

E-mail: fzmbi@public.fz.fj.cn

Telephone: +86 591 3732741, Fax: +86 591 3720685

URL: <http://www.maximbio.com/phdcnlibrary.htm>

Produce the EZInet™ phage display cDNA library kit which utilizes filamentous phage, M13, to express foreign peptides or proteins.

Goodwin Biotechnology Incorporated

1850 NW 69th Ave., Plantation, FL 33313, USA

Telephone: (954) 321-5300, Fax: (954) 587-6378

E-mail: gbi@gate.net

URL: <http://www.gate.net/~gbi>

Monoclonal antibodies produced from ascites production in mice and hollow fiber bioreactors. Specializes in human cytomegalovirus (HCMV), HSV1 and 2, HIV-1.

Harlan Bioproducts for Science, Inc.

P.O. Box 29176

Indianapolis, IN 29176, USA

Telephone: (317) 894-7536, Toll free: (800) 972-4362, Fax: (317) 894-1840

E-mail: hbps@harlan.com

URL: <http://www.harlan.com/hbps>

Specialize in the custom production of polyclonal and monoclonal antibodies, *in vivo* and *in vitro*. Offer cGMP and non-cGMP production, hybridoma development, MAP testing, purification, endotoxin testing and other immunological services.

Heraeus Instruments Inc.

111 A Corporate Boulevard, South Plainfield, NJ 07080, USA

Telephone: (908) 754-0100, Fax: (908) 754-9494

E-mail: custservice@heraeus-inst.com

URL: <http://www.heraeus-inst.com>

Developed the miniPERM Bioreactor for culture of hybridoma cells. Small size bioreactor designed for high density culture. Other cell culture products include incubators, cell culture vessels, and continuous flow centrifuges.

IDEC Pharmaceuticals Corp.

11011 Torreyana Rd.

San Diego, CA 92121, USA

Telephone: (619) 550-8500, Fax: (619) 550-8750

E-mail: info@idecpharm.com

URL: <http://www.idecpharm.com> (under construction)

Research and development of monoclonal antibodies. Phage display technologies investigated. Alternative adjuvant ProVax used in cancer studies. Large scale antibody production.

ImmuCell Corp.

56 Evergreen Dr., Portland, ME 04103-1066, USA

Telephone: (207) 878-2770, Fax: (207) 878-2117

Develop and manufacture cow milk-derived passive antibody products. Rabbits and cows used for polyclonal antibody production. Tissue cultures used in vaccine development. Applications include a product to prevent scours in calves and others to prevent human gastrointestinal infections.

Institute for Animal Science and Health (ID-DLO)

Research Head Office

Edelhertweg 15, Postbus 65, 8200 AB Lelystad, The Netherlands

Telephone: +31 320 238238 Fax: +31 320 238050 (from U.S. 31- 320238050)

email: postkamer@id.dlo.nl

URL: <http://www.agro.nl/iddlo/welcome.htm>

Descriptors: Manufacture Specol, a water-in oil emulsion used as an alternative to Freund's adjuvant for research purposes. Produce monoclonal antibodies specific for bovine, porcine and avian immunoglobulins, and a wide range of animal vaccines and diagnostic test kits.

Institute of Laboratory Animal Science

University of Zurich

Winterthurerstrasse 190, 8057 Zurich, Switzerland

Telephone: +41 1 257 54 52, Fax: +41 1 257 57 03 (from U.S. 41-1257575703)

e-mail: frhom@ltk.unizh.ch

URL: <http://www.unizh.ch/labtier>

Generate MAB in various sized hollow fiber bioreactors and roller bottles. Hybridoma lines can be sub-cloned to increase yields if necessary. Customers receive the required amount of MAB as a crude bioreactor harvest. FCS-free culture available if the hybridoma line has previously been adapted to this condition by the customer.

Integra Biosciences (Multiple Addresses)

P.O. Box 74 , CH-8304, Wallisellen, Switzerland

Telephone: (01) 830 22 77, Fax: (01) 830 78 52

Ruhberg 4, D-35463 Fernwald, Germany

Telephone: (44) 6404 809 0, Fax: (44) 6404 58 65

15, Route de Montmorency, B.P. 74, F-95603 Eaubonne Cedex, France

Telephone: (33) 1 39 59 84 42, Fax: (33) 1 39 59 19 20

Ascot Industrial Estate, Unit 9, Ickneild Way, Letchworth, Herts, SG6 1TD, United Kingdom

Telephone: (44) 1462 48 65 48, Fax: (44) 1462 48 66 78

44 Stedman Street, Suite 2, Lowell, Massachusetts 01851, USA

Telephone: 1 (800) 886-8675, Fax: 1 (508) 934-9888

E-mail: INTEGRA_Biosciences@freeway.de

URL: http://www.freeway.de/INTEGRA_Biosciences

Offer for sale, TECNOMOUSE, a hollow fiber "in-vitro" system developed for the cultivation of cells and the expression of cell products. Optimal media utilization, direct oxygenation and yields of up to 500 mg of monoclonal antibody per month possible. Allows up to five different cell lines to be cultivated simultaneously. An alternative to the production of monoclonal antibodies in mice.

INTEGRA CELLline, a compartmentalized cell culture system based on a T-flask design. This system can cultivate up to 10^8 cells/ml and produce 100 mgs of monoclonal antibody in a month. This membrane design greatly reduce serum, media and time consuming purification steps. It is available in 3 sizes, disposable , efficient, and cost effective.

Lampire Biological Laboratories, Inc.

P.O. Box 270, Pipersville, PA 18947, USA

Telephone: (215) 795-2838, Fax: (215) 795-0237

E-mail: lampire@lampire.com

URL: <http://www.lampire.com>

Develops and produces monoclonal and polyclonal antibodies. Uses ascites production and bioreactor technologies. Catalog available. Monoclonal animal models include mice, rats, and hamsters. Polyclonal animal models are rabbits, chickens, goats, sheep, and donkeys. Custom antibodies can be designed and produced using rats, cows, guinea pigs, and horses.

Lazar Research Laboratories, Inc.

731 North La Brea Avenue Suite 5

Los Angeles, CA 90038, USA

Telephone: (213) 931-1204 or 001-213- 931-1204 (outside U.S.), Toll free: (800) 824-2066,

Fax: (213) 931-1434 (outside U.S. and Canada, dial 001 country code before the Telephone number)

E-mail: service@lazarlab.com

<http://www.lazarlab.com/biotech.htm>

Offer the Ultra M Micro Bioreactor System for sale. Can convert standard six well cultura plate or T-flasks into a continuous flow bioreactor system.

Maine Biotechnology Services, Inc.

383 Presumpscot St., Portland, ME 04103, USA

Telephone: (207) 773-1993, Fax: (207) 773-1163

E-mail: mabsbymbbs@aol.com

Produces customized monoclonal and polyclonal antibodies for diagnostic companies and researchers. Several hundred antibodies are listed in the catalog. Bioreactors and mice are used for monoclonal production. Polyclonal antibodies are produced from sheep, goats, and rabbits.

National Institute of Public Health and the Environment (RIVM)

Laboratory for the Control of Biological Products (LCB)

P.O. Box 1, 3720 BA, Bilthoven, The Netherlands

Telephone: +31 30 2743697, Fax: +31 30 2744420 (from the U.S. 31-302744420)

E-mail: Mia.Leerling@RIVM.nl

Available to provide information on in vitro production of monoclonal antibodies. Knowledge of cell culture, roller bottle, and hollow fiber system technologies.

New England Biolabs, Inc.

32 Tozer Road

Beverly, MA 01015, USA

Telephone: (508) 927-5054, Toll free: (800) 632-7799, Fax: (508) 921-1350

E-mail: info@neb.com

URL: <http://www.neb.com/neb/company/company.html>

Offer phage display peptide library kits displayed on bacteriophage M13.

Office of Biotechnology**Iowa State University**

Ames, Iowa 50011-3260, USA

Telephone: (515) 294-2472, Fax: (515) 294-0453

E-mail: kharkins@iastate.edu or clap@iastate.edu

<http://biotech.zool.iastate.edu/Facilities/cellhyb/homepage.htm>

Offer bulk monoclonal antibody production in bioreactors or ascites production in mice.

Paul-Ehrlich-Institut**Federal Agency for Sera and Vaccines**

Department of Veterinary Medicine

Paul-Ehrlich-Straße 51-59

D-63225 Langen Neurott, Germany

Telephone: (49) 6103-77/77-0, Fax: (49) 6103/77-123 (from U.S. 49-610377123)

E-mail: peiyup@em.uni-frankfurt.de

URL: <http://www.rz.uni-frankfurt.de/~peiski>

Research conducted on alternative adjuvants for vaccine development and research immunizations with little side effects.

QED Bioscience

11021 Via Frontera #203, San Diego, CA 92127, USA

Telephone: (619) 675-2405, Toll free: (800) 929-2114 or , Fax: (619) 592-1509

URL: <http://www.qedbio.com>

Develops and supplies monoclonal and polyclonal antibodies to an extensive range of antigens for research laboratories and healthcare industry. Offers custom antibody development (monoclonal and polyclonal), ascites production, and in vitro antibody production services. New products include culture media for hybridomas, immunoassay kits, ascites production kits, and hybridoma development kits.

Ribi ImmunoChem Research, Inc.

553 Old Corvallis Road, Hamilton, MT 59840, USA

Telephone: (406) 363-6214, Toll free: (800) 548-7424, Fax: (406) 363-6129

E-mail: general@ribi.com

Manufactures the Ribi Adjuvant System (RAS). RAS is appropriate for use in a variety of laboratory animals such as mice, rats, guinea pigs, rabbits, goats and non-human primates.

Independent comparative studies have shown that RAS is an effective adjuvant without the toxic side effects associated with the use of Freund's adjuvant.

Scantibodies Laboratory, Inc.

9336 Abraham Way

Santee, CA 92071, USA

Telephone: (619) 258-9300, Fax: (619) 258-9366

E-mail: n/a

URL: <http://www.biospace.com> (search under companies)

Offer custom in vitro monoclonal antibody production using a variety of bioreactors. Ascites production in mice.

Spectrum® U.S. Headquarter

23022 La Cadena Drive

Laguna Hills, CA 92653-1352, USA

E-mail: techsupport@spectumlabs.com

Telephone: (714) 58-3500 or toll free 1-(800) 654-0111, Fax: (714) 855-6120

URL: <http://www.spectumlabs.com>

Spectrum® has merged with Cellco, Inc. (see Cellco), makers of an artificial capillary system for in vitro production of monoclonal antibodies, proteins, and viruses. Spectrum is a supplier of dialysis and ultrafiltration membranes used for biomedical research, molecular biology, and clinical diagnostics.

Serologicals Corp.

780 Park North Blvd., #110, Clarkston, GA 30021, USA

Telephone: (404) 296-5595, Fax: (404) 296-0052

Provides monoclonal and polyclonal antibodies to pharmaceutical companies that develop diagnostics. Locate and recruit antibodies for use as test kit controls. Antibodies are human subject derived such as rabies immunoglobulin and hepatitis A and B.

Sierra BioSource, Inc.

1180-C Day Rd., Gilroy, CA 95020-9308, USA

Telephone: (408) 847-5444, Fax: (408) 847-5044

E-mail: antibody@garlic.com

Produces cell lines for clients. Uses some in vitro techniques for monoclonal antibody production. Monoclonal models include mice, rats, and humans. Polyclonal antibodies produced on demand from rats, rabbits, and guinea pigs.

TNO Prevention and Health**Immunological and Infectious Diseases**

P.O. Box 2215, 2301 CE, Leiden, The Netherlands

Telephone: +31.71.518.1431, Fax: +31.71.518.1901 (from U.S. 31-715181901)

E-mail: nd.zegers@pg.tno.nl

Offer complete services to obtain suitable site-specific poly- and monoclonal antibodies using antigen or synthetic peptides including: selection of epitopes, synthesis of peptides, construction of peptide conjugates for immunization, and development of selection assays. MAbs are produced using in vitro methods.

Unisyn Technologies Inc.

25 South St., Hopkinton, MA 01748, USA

Telephone: (508) 435-2000, Toll free: (800)-4UNISYN, Fax: (508) 435-8111

E-mail: unisyn@unisynth.com

URL: <http://www.unisynth.com>

Manufacture hollow fiber bioreactor instruments and accessories and protein purification products for monoclonal antibodies and recombinant protein production. GMP contract service facility for clients on a research or full outsourced basis.

Zonagen, Inc.

2408 Timberloch Place, B4

The Woodlands, TX 77380, USA

E-mail: zonasci@infohwy.com

Telephone: (281) 367-5892 or toll free 1-888-448-4837, Fax: (281) 363-8796

Producers of Z-maxTM and ImmuMax^{SR}, alternatives to Freund's adjuvants. Z-maxTM was designed specially for use with HIS tagged recombinant proteins, while ImmuMax^{SR} was developed to elicit lasting titers to both native and recombinant proteins as well as synthetic peptides.

Educational Web Site Resources

Efforts have been made to cite sources in a consistent style and to ensure accurate Uniform Resource Locator (URL) addresses. However given the variable nature of information available on the internet, citations may vary in their format and URL addresses may be subject to change.

General Resources

The John Hopkins CAAT Alternatives to Animal Testing Website

<http://www.sph.jhu.edu/~altweb/> (accessed June 20, 1997). A project team of university, government, nonprofit, and company organizations working together to create a comprehensive global resource on alternative methods.

Nature America Inc. 1995 [copyright] **Nature Buyers' Guide**. <http://www.guide.nature.com/> (accessed June 9, 1997). Searchable guide containing address information, along with a listing of products, services, and trade names of more than 1700 companies in the natural sciences marketplace. More than 1100 products and services listed.

Shreder, Kevin. **Antibody Resource Page**. <http://www.antibodyresource.com> (accessed June 11, 1997). Comprehensive web page on antibodies with links to educational resources, databases and databanks (hybridoma banks included), journals, companies, information on specific antibodies and more. Terrific site to begin a search.

Smith, Cynthia, D'Anna Jensen, et al. March 1997 **Information Resources for Adjuvants and Antibody Production: Comparisons and Alternative Technologies** <http://www.nal.usda.gov/awic/pubs/antibody.htm> (accessed June 11, 1997). Electronic version of this document. Contains over 500 current bibliographic citations regarding adjuvants and antibody production methods compiled from scientific journals, proceedings, and newsletters. A company/institute listing of suppliers of antibodies and antibody production products are also listed. Emphasis placed on citing comparative studies and research into alternative methods.

Articles and References

- Cronin, M.E., S.T. Jones, et al. March 11, 1995 [last update]. **Antibody Engineering**.
<http://www.nimr.mrc.ac.uk/CC/ccaewg/phgedisp.htm> (accessed June 11, 1997). Overview of the history of human antibody engineering technologies developed by researchers associated with the United Kingdom Medical Research Council (MRC) Collaborative Centre.
- Czirbik, Rudolf J., Steven M. Rosen, et al. **Factors affecting antibody production efficiency in hollow-fiber bioreactors**. Published in *IVD Technology Magazine* in July, 1996.
<http://www.devicelink.com/ivdt/archive/96/07/007.html> (accessed June 11, 1997).
- De Young, H. Garrett **New Antibody Technologies on Tap?** Published in *The Scientist* 10(18):20, September 16, 1996.
http://www.the-scientist.library.upenn.edu/yr1996/sept/tools_a_960916.html (accessed May 8, 1997).
- Dyax corporation receives third fundamental U.S. patent covering phage display technology**. November 21, 1996 [press release date].
http://www.fpinc.com/Release_Comp/Releases/DYAX/311-21-96.html (accessed May 8, 1997). Press release from Dyax Corporation.
- Haak-Frendscho, Mary. **Why IgY? Chicken polyclonal antibody, an appealing alternative**. Published in *Promega Notes Magazine* Number 46, 1994, p.11.
<http://www.promega.com/pnotes/46/2259e/2259e.html> (accessed June 11, 1997).
- Monoclonal antibody technology**. Excerpted from "What is Biotechnology?" Washington, D.C.: Biotechnology Industry Organization, 1989.
<http://esg-www.mit.edu:8001/bio/imm/monoclonal.html> (accessed June 11, 1997). Easy to read overview of monoclonal antibody technology.
- Pam Pimpa. January 1997 [last update]. **Monoclonal antibody production by 6bb hybridoma in batch and fed-batch cultures**. Department of Agricultural Science and Technology Faculty of Applied Sciences, King Mongkut's Institute of Technology, North Bangkok, Bangsue, Bangkok, Thailand. <http://library.kmitnb.ac.th/article/atc00037.htm> (accessed May 8, 1997).
- Sauceda, Jo Ann. **Overview of immunology and the production of monoclonal antibodies**. Department of Biology at Texas AandM University, Kingsville, Texas.
<http://ntri.tamuk.edu/monoclonal/monoclonal.html> (accessed June 17, 1997).
- van der Kamp, Margot and Wim de Leeuw **Short review of in vitro production methods for monoclonal antibodies**. Published in *NCA Newsletter* 3, October 1996.
<http://www.pdk.dgk.ruu.nl/nca/> (accessed June 27, 1997).

Faculty Home Pages

Dessertation abstracts. Department of Immunotechnology, Lund University, Lund, Sweden. <http://www.kc.lth.se/immun/TEXTER/Diss-abstr.html> (accessed June 17, 1997). Dissertation abstracts from doctoral theses published from graduate students at Lund University on antibody production. Links provided to student home pages for further information on individual research interests and publications.

Georgiou, George. July 12, 1996 [last updated] Department of Chemical Engineering, University of Texas at Austin, Austin, Texas. <http://www.che.utexas.edu/grad-brochure/georgiou.html> (accessed June 17, 1997). Research interests in the function and engineering of antibodies, including the production of complex pharmaceuticals in bacteria.

Kay, Brian K. December 18, 1996 [last update] Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina.. <http://www.unc.edu/depts/biology/kay.html> (accessed June 17, 1997). Research interests in macromolecular complexes of differentiating cells. Use of M13 bacteriophage-display of random peptides and proteins to study protein-protein interactions. Links provided to "Webpage Companion to Phage Display Book."

Makowski, Lee. Institute of Molecular Biophysics, Biochemistry Division, Florida State University, Tallahassee, Florida. <http://www.sb.fsu.edu/~makowski> (accessed June 17, 1997). Research interests in the structures, activity, and engineering of biomolecular systems. Current research areas include the study of filamentous bacteriophages.

Courses Offerred on Phage Display Technology

Barbas, Carlos, Dennis Burton, and Gregg Silverman. **Phage display of combinatorial antibody libraries. November 4-17, 1997.** http://edclio.cshl.org/meetings/97c_mac.htm (accessed June 17, 1997). Library/lecture course focusing on the construction of combinatorial antibody libraries from immune and non-immune sources, as well as synthetic antibody libraries.

Rathod, Pradipsinh K., Course Director **Combinatorial chemistry, phage display and in vitro evolution. May 5-9, 1997** Center for Advanced Training in Cell and Molecular Biology (CATMC), Department of Biology, The Catholic University of America, Washinton, D.C. <http://www.cua.edu/www/catc> (accessed June 17, 1997). Introduction to different types of combinatorial libraries.

Conferences

International Business Communications (IBC) Southborough, MA. <http://www.ibcusa.com/> (accessed June 27, 1997). An independent organizer that sponsors conferences on antibody engineering, phage display technologies, and other science related topics. Proceedings of previous conferences available for a fee.

Larrick, J.W.¹ and Dennis Burton², organizers. **Therapeutic Antibody Technology 1997** Palo Alto Institute of Molecular Medicine, Mountain View, CA¹ and Scripps Research Institute, La Jolla, CA². http://www.antibody_resource.com/therapeutic-antibody.html (accessed June 27, 1997). Researchers in the field of recombinant therapeutic monoclonal antibodies will present recent work in this rapidly changing field.

Organizations

NORTH AMERICAN RESOURCES:

Alternatives Research and Development Foundation

14280 Golf View Drive
Eden Prairie, Minnesota 55346, USA

TELEPHONE: (612) 949-2409

FAX: (612) 949-2619

E-MAIL: ARDFJM@minn.net

WORLD WIDE WEB: http://www.aavs.org/AAVS_ARDFfund.html

CONTACT: John McArdle, Ph.D., Director

TYPE OF INSTITUTION/ORGANIZATION: Private, nonprofit

RESOURCES/SERVICES: Developed to support alternatives to the use of animals in biomedical research, testing, and education. Previously known as the Demeter Fund. Grants available to scientists and educators. Projects that have been funded include basic research into the use of human cells to produce monoclonal antibodies, in vitro culturing of monoclonal antibodies, and the development of a simple kit to replace ascites production. Other services include publications, lectures, seminars and workshops.

REQUESTOR: Anyone.

COSTS: none.

American Society for the Prevention of Cruelty to Animals (ASPCA)

Alternatives Directory
424 East 92nd Street
New York, New York 10128-6804, USA

TELEPHONE: (212) 876-7700 ext. 4401

FAX: (212) 348-3031

E-MAIL: stephenz@aspca.org

URL: <http://www.asPCA.org>

CONTACT: Stephen Zawistowski

TYPE OF INSTITUTION/ORGANIZATION: Private, nonprofit

RESOURCES/SERVICES: Provide an eighteen page booklet entitled *Three R Alternatives: An International Directory of Funding Sources* written to help scientists identify potential funding sources for work involving refinement, reduction, or replacement of animal experiments. Funding sources include animal protection organizations, other charities, private industry, universities, and government agencies from all over the world. Some sources listed have funded research into alternative antibody production methods.

REQUESTOR: Anyone.

COSTS: \$10 for Alternatives directory, inquire about discounts for bulk orders.

Animal Welfare Information Center (AWIC)

Agricultural Research Service
National Agricultural Library
10301 Baltimore Avenue
Beltsville, Maryland 20705, USA

TELEPHONE: (301) 504-6212

FAX: (301) 504-7125

E-MAIL: awic@nal.usda.gov

URL: <http://www.nal.usda.gov/awic>

CONTACT: Jean Larson, Coordinator

TYPE OF INSTITUTION/ORGANIZATION: Public, nonprofit, government agency

RESOURCES/SERVICES: Vast collection of serials, monographs, and audiovisuals within the National Agricultural Library (NAL). Documents may be borrowed through an interlibrary loan. For more information on document delivery, contact (301) 504-5755. The Center performs brief complimentary searches of AGRICOLA and other relevant databases. The Center can also assist you in formulating your own database searches, provides conference facilities and host training sessions, and can make available speakers and/or a tabletop exhibit for training sessions, conferences, and workshops. The Center produces bibliographies on topics such as animal care, analgesia, animal testing alternatives, training materials, and other relevant topics to animal welfare. Publishes the *Animal Welfare Information Center Newsletter*.

REQUESTOR: Anyone.

COSTS: All publications are available for free; literature searches on a cost recovery basis; NAL may charge for certain services such as providing photocopies, document delivery, etc.

Canadian Association for Laboratory Animal Science L'Association Canadienne Pour La Technologies des Animaux de Laboratoire

c/o CALAS National Office
Biosciences Animal Service
University of Alberta
Edmonton, Alberta T6G 2E9, CANADA

TELEPHONE: (403) 492-5193

FAX: (403) 492-7257

E-MAIL: dmckay@gpu.srv.ualberta.ca

URL: <http://www.utoronto.ca/calas/>

CONTACT: Donald McKay, Ph.D.

TYPE OF INSTITUTION/ORGANIZATION: Professional, nonprofit

RESOURCES/SERVICES: Produces educational materials, videos, and a monthly newsletter (CALAS/ACTAL Newsletter). Hold annual meetings with workshops, seminars, and poster sessions. Antibody production techniques and issues periodically addressed in newsletter and discussed at meetings.

REQUESTOR: Laboratory animal professionals.

COSTS: Vary according to materials.

Canadian Council on Animal Care (CCAC)

350 Albert Street, Suite 315

Ottawa, Ontario K1R 1B1, CANADA

TELEPHONE: (613) 238-4031

FAX: (613) 238-2837

E-MAIL: lroach@bart.ccac.ca

CONTACT: Dr. James Wong, Director of Assessments

TYPE OF INSTITUTION/ORGANIZATION: Private, nonprofit

RESOURCES/SERVICES: Establishment and enforcement of standards and guidelines (in Canada) concerning the use of animals in research, testing and teaching. Including written recommendations on immunization procedures. Maintain active, expert committees on all aspects of animal care and use. The Council's program is based on its major publication "Guide to the Care and Use of Experimental Animals," Volume 1, 2nd Edition (1993) and Volume 2 (1984). CCAC conducts workshops and training courses on various aspects of the care and use of experimental animals, as well as the training of personnel working with these animals. The Council addresses alternative methods and conducts a course on tissue culture. Semi-annually publishes the newsletter, *Resource*.

REQUESTOR: Anyone.

COSTS: Vary according to materials.

Division of Animal Welfare

Office for Protection from Research Risks (OPRR)

National Institutes of Health

6100 Executive Boulevard, Suite 3B01, MSC-7507

Rockville, Maryland 20892-7507, USA

TELEPHONE: (301) 496-8101 x233

FAX: (301) 402-0527

E-MAIL: CS19N@NIH.GOV

URL: <http://www.nih.gov:80/grants/oprr/oprr.htm>

CONTACT: Nelson L. Garnett, D.V.M., Director

TYPE OF INSTITUTION/ORGANIZATION: Government

RESOURCES/SERVICES: The Office for Protection from Research Risks (OPRR) fulfills responsibilities set forth in the Public Health Service (PHS) Act. The Division of Animal Welfare directs the development, implementation, and compliance oversight activities for the PHS Policy on Humane Care and Use of Laboratory Animals. Services provided include educational and instructional programs as well as the production of resource materials relating to the responsibilities of the research community for the appropriate care and use of laboratory animals. OPRR Animal Welfare Report Number Report 95-02 addresses issues of assurance when using sources of custom made antibodies. Co-sponsored a workshop entitled *Alternatives to Monoclonal Antibody Production* conducted on September 24-25, 1997, in Baltimore, Maryland. Proceedings will be printed at a later date.

REQUESTOR: Anyone.

COSTS: Registration fees for workshops and fees for publications vary according to materials.

Institute of Laboratory Animal Resources (ILAR)

National Academy of Sciences
2101 Constitution Avenue, N.W.
Washington, D.C. 20418, USA

TELEPHONE: (202) 334-2590

FAX: (202) 334-1687

E-MAIL: twolfle@nas.edu

URL: <http://www2.nas.edu/ilarhome/>

CONTACT: Tom Wolfle, D.V.M. , Ph.D., Director

TYPE OF INSTITUTION/ORGANIZATION: ILAR is a unit of the National Research Council's (NRC) Commission on Life Sciences (CLS). The NRC is the working arm of the National Academy of Sciences (NAS), a private, non-governmental, non-profit organization.

RESOURCES/SERVICES: Produce *ILAR NEWS* - a quarterly journal, available free-of-charge to institutional animal care and use committees, scientists, and veterinarians. Volume 37, number 3, 1995, dedicated entirely to papers on adjuvants and antibody production. Publishes guidelines that assist in the implementation of national policies or laws. Maintains an information database published as *Animals for Research: A Directory of Sources* that assists scientists in locating specific animals and models.

REQUESTOR: Anyone.

COSTS: Vary according to materials.

The Johns Hopkins Center for Alternatives to Animal Testing (CAAT)

111 Market Place, Suite 840
Baltimore, Maryland 21202-6709, USA

TELEPHONE: (410) 223-1693

FAX: (410) 223-1603

E-MAIL: CAAT@CAAT.spharbor.jhu.edu

URL: <http://infonet.welch.jhu.edu/~caat>

CONTACT: Alan M. Goldberg, Ph.D., Director

TYPE OF INSTITUTION/ORGANIZATION: University, nonprofit

RESOURCES/SERVICES: Programs focus on laboratory research, education and information, and validation of alternative methodologies. Administer a grants program to provide funds for scientists to develop alternatives to whole animals for product safety and drug efficacy testing. Publications include *Center for Alternatives to Animal Testing* newsletter, *CAATALYST*- a newsletter for middle school students, meeting proceedings, and technical reports. CAAT is also the primary sponsor of the Alternatives to Animal Testing Website located at <http://www.sph.jhu.edu/~altweb>

REQUESTOR: Anyone

COSTS: Vary according to materials.

Public Responsibility in Medicine and Research (PRIM&R)

Fourth Floor

132 Boylston Street

Boston, Massachusetts 02116, USA

TELEPHONE: (617) 423-4112

FAX: (617) 423-1185

E-MAIL: PRMR@aol.com

URL: <http://www.aamc.org/research/primr/>

CONTACT: Joan Rachlin, Executive Director

TYPE OF INSTITUTION/ORGANIZATION: Private, nonprofit

RESOURCES/SERVICES: Conducts conferences on a broad range of topics including ; Institutional Review Boards (IRBs), Institutional Animal Care and Use Committees (IACUCs), and Healthcare Ethics Committees (HCECs). Publish proceedings and educational materials. Workshop entitled *Special Problems When Using Immunological Technologies and Antibody Production* presented at March 1997 *Animal Care and Use in a Changing Research Environment: Ethics, Technology, Accountability, and Efficiency* meeting.

REQUESTOR: Anyone.

COSTS: Vary according to materials.

Scientists Center for Animal Welfare (SCAW)

Golden Triangle Building One

7833 Walker Drive, Suite 340

Greenbelt, Maryland 20770, USA

TELEPHONE: (301) 345-3500

FAX: (301) 345-3503

E-MAIL: scaw@erols.com

CONTACT: Lee Krulisch, Executive Director

TYPE OF INSTITUTION/ORGANIZATION: Private, nonprofit

RESOURCES/SERVICES: Publishes newsletter, conference proceedings, training manuals, and disseminates materials from other organizations. Antibody production issues addressed at several conferences.

REQUESTOR: Anyone.

COSTS: Some services are free, others are fee-for-services basis.

TUFTS University Center for Animals and Public Policy

School of Veterinary Medicine

200 Westboro Road

North Grafton, Massachusetts 01536, USA

TELEPHONE: (508) 839-7991

FAX: (508) 839-2953

E-MAIL: arowan@opal.tufts.edu

URL: <http://www.vec.tufts.edu:80/vetadmissions/mspubpolicy.html>

CONTACT: Andrew Rowan, PhD., Director

TYPE OF INSTITUTION/ORGANIZATION: University, nonprofit

RESOURCES/SERVICES: Focus on programs dealing with the ethical, legal, scientific, and social issues relating to the status of animals in society. Interest in analyzing refinements in experimental techniques including antibody production methods, to reduce animal distress. Offer a Master of Science degree in animals and public policy. Publications include *The Alternatives Report*, *Center for Animals* newsletter, proceedings, and reports.

REQUESTOR: Anyone.

COSTS: Nominal fees for newsletter and proceedings.

UC Center for Animal Alternatives

School of Veterinary Medicine

University of California, Davis

Davis, California 95616-8684, USA

TELEPHONE: (916) 752-1800

FAX: (916) 754-8608

E-MAIL: cjhubbard@ucdavis.edu

URL: <http://www.vetmed.ucdavis.edu/CenterforAnimals/main.htm>

CONTACT: Lynette A. Hart, PhD., Director

TYPE OF INSTITUTION/ORGANIZATION: University, nonprofit

RESOURCES/SERVICES: Gather and disseminate information on animal alternatives to be used by the University of California campuses and California academic libraries. Develop resources to access the literature of alternatives, present at conferences and workshops, and publish the *UC Alert: Newsletter*. Publish a student fact sheet *The Mouse in Science* that describes the use of mice in monoclonal antibody production.

REQUESTOR: Primarily researchers and IACUC (Institutional Animal Care and Use Committee) members.

COSTS: Respond to inquires without charge, but this does not include and extensive literature search. Request a contribution to cover publishing and mailing costs of newsletter.

EUROPEAN and AUSTRALIAN RESOURCES:

Australian and New Zealand Council for the Care of Animals in Research and Teaching, Limited (ANZCCART)

P.O. Box 19
Glen Osmond SA 5064
AUSTRALIA

P.O. Box 598
5064 Wellington
NEW ZEALAND

TELEPHONE: 61-08-303-7393 (Australia)

64-04-472-7421(New Zealand)

FAX: 61-08-303-7113 (Australia)

64-04-473-1841(New Zealand)

E-MAIL: anzccart@waite.adelaide.edu.au

URL: <http://www.adelaide.edu.au/ANZCCART/>

CONTACT: R.M. Baker

TYPE OF INSTITUTION/ORGANIZATION: Private, nonprofit

RESOURCES/SERVICES: Quarterly newsletter, and other publications on euthanasia, animal care and use committees, wellbeing of research animals, alternatives for undergraduate education, laboratory animal surveys, tumour cell lines available in Australia, humane care and use of animals in research, and animal pain. Issues concerning adjuvant use and antibody production periodically addressed in newsletter.

REQUESTOR: Anyone.

COSTS: Vary according to materials.

European Center for Validation of Alternative Methods (ECVAM)

European Center for the Validation of Alternative methods
TP 580, JRC Environment Institute
21020 Ispra (VA), ITALY

TELEPHONE: +39 332 785996

FAX: +39 332 785336

E-MAIL: julia.fentem@ei.jrc.it or michael.balls@jrc.it

URL: <http://www.ei.jrc.it/ecvam/>

CONTACT: Dr. Michael Balls, PhD., Director

TYPE OF INSTITUTION/ORGANIZATION: ECVAM was established by the European Union (EU) to specifically coordinate the validation of alternative test methods at the European Union level. It's origins are based on Directive 86/609/EEC, an European Union law containing language encouraging research into the development and validation of alternative techniques which reduce, refine, and replace the use of laboratory animals.

RESOURCES/SERVICES: Provide information on: alternative methods and their status; validation study protocols; chemical formulations; and the development of a register of European in vitro pharmacotoxicologists. Conduct workshops, symposia, and assign task forces

to study particular issues. Results on workshop reviewing current monoclonal antibody production techniques to be published in April, 1997 issue of *ATLA* journal.

REQUESTOR: Anyone, but primarily scientists, administrators, and government officials.

COSTS: None for individuals requesting only a single or few copies of publications.

**Institut für Labortierkunde der Universität Zürich
Institute of Laboratory Animal Science**

University of Zurich
Winterthurerstrasse 190
8057 Zurich, SWITZERLAND

TELEPHONE: +41-(0)1- 257-11-11 or +41-(0)1-257-54-51 (from the U.S. 41-1257575703)

FAX: +41-(0)-1-257-57-03

CONTACT: Prof. Dr. med. vet. Peter E. Thomann, Director

URL: <http://www.unizh.ch/labtier/>

TYPE OF INSTITUTION/ORGANIZATION: University

RESOURCES/SERVICES: Provides classes and training courses for technicians, students and postgraduates. Breeds rats and mice, offers diagnostic services for rodents and rabbits, offers in vitro production of monoclonal antibodies, and operates a consulting service to answer questions relating to the care of laboratory animals. Most information on Web site is in German.

REQUESTOR: Laboratory animal users.

COSTS: Vary according to material.

Netherlands Centre Alternatives to Animal Use (NCA)

Yalelaan 17
NL-3584 CL Utrecht
THE NETHERLANDS

TELEPHONE: 31 30 (2)532186

FAX: 31 30 (2)539227

CONTACT: Margot D.O. Van Der Kamp, Secretariat

E-MAIL: m.vanderkamp@cc.ruu.nl

URL: <http://www.pdk.dgk.ruu.nl/nca/>

TYPE OF INSTITUTION/ORGANIZATION: Established in 1994 to provide a central and independent body to stimulate development, validation, acceptance, and employment of alternative methods. The NCA is funded by the Dutch Alternatives to Animal Experiments Platform and Utrecht University.

RESOURCES/SERVICES: Establish expert working groups including groups on vaccines and immunoglobulins, give lectures on alternatives, and publish *Netherlands Centre Alternatives to Animal Use Newsletter*.

REQUESTOR: Anyone.

COSTS: newsletter free

Universities Federation for Animal Welfare

8 Hamilton Close
South Mimms, Potters Bar,
Hertfordshire, EN6 3QD, UNITED KINGDOM

TELEPHONE: 01707-658202

FAX: 01707-649279

E-MAIL: trevor.poole@ucl.ac.uk

URL: <http://www.users.dircon.co.uk/~ufaw3/>

CONTACT: Victoria Taylor, Development Officer

TYPE OF INSTITUTION/ORGANIZATION: Private, charitable

RESOURCES/SERVICES: Publications, reprints, videos, educational brochures, and advisory services. Publishes a quarterly scientific journal entitled *Animal Welfare*, which brings together information from zoos, laboratories, farms, wild, and companion animals. Publication of refinement techniques encouraged.

REQUESTOR: Anyone.

COSTS: Vary according to materials. Annual subscriptions to the journal *Animal Welfare* cost £50/US\$100.

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Document Delivery Services Branch, PhotoLab
10301 Baltimore Ave., NAL Bldg.
Beltsville, Maryland 20705-2351

FAX: 301-504-5675
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E-mail: lending@nal.usda.gov

Contact the Head, Document Delivery Services Branch at (301) 504-5755 or via Internet at ddsbhead@nal.usda.gov with questions or comments about this policy.

National Agricultural Library Document Delivery Services

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For postal service delivery send requests to:

USDA National Agricultural Library
Document Delivery Services Branch, ILL
10301 Baltimore Ave., NAL Bldg...
Beltsville, Maryland 20705-2351

For electronic access and delivery :

The Document Delivery Services Branch accepts ILL requests from libraries via several electronic methods. All requests must comply with established routing and referral policies and procedures. A sample format for ILL requests follows.

(over)

INTERNET.....lending@nal.usda.gov

Start the subject line with one word format:

3 letter month abbreviation + day + NAL + # of request placed that day,

For Example: jul25NAL4 (if this is the fourth request on July 25).

For Example: jul 25NAL1-4 (if this is four requests submitted at one time)

OCLC.....AGL

TELEFACSIMILE.....301-504-5675

Requests can be created on standard ILL forms and then faxed. NAL will fill via FAX at no additional cost if FAX number is included on request. NAL will send up to 30 pages per article. If request exceeds 30 pages, NAL will ship via postal service. There is no RUSH service.

ARIEL™198.202.222.162

NAL will fill the request via ARIEL™ if the ARIEL™ address is included in the request. NAL treats ARIEL™ at an alternative delivery mechanism--not an expedited service. NAL will send up to 30 pages per article via ARIEL™. If request exceeds 30 pages, NAL will ship via postal service.

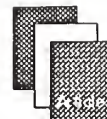
Required data elements for all requests:

- Complete mailing information for all requests regardless of method of delivery.
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- Copyright Compliance – Pre-printed forms must contain your signature to indicate copyright compliance. On e-mail requests include the complete Statement of Copyright Compliance. Libraries may indicate compliance by including the initials of one statement, either "CCL" for compliance with the copyright law or "CCG" for compliance with Copyright Guidelines or a statement that the request complies with U.S. Copyright Law or other acceptable copyright laws (i.e. IFLA, CLA, etc.). Libraries must also provide authorizing official's name. Requests will be rejected if this information is not included.
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Sample Electronic Mail Request

(Your Institutions Name)/NAL	JUL25NAL1	Date Not Needed After: 9/25/97
(Your Department or Office Your University Library or Institution City, State or Province, Country, Mail Code)		
Dr. Smith (patron name) Biology Dept. (patron office) Canadian Journal of Soil Science 1988 v 68(1): 17-27 (complete citation) De Jong, R. Comparison of two soil-water models under semi-arid growing conditions		
NAL Call Number: 56.8 C162 Ver: AGRICOLA		
Remarks: Not available at university or in region		
Authorized by: Charles Johnson		CCL Maxcost: \$15.00
(your) Phone# (301)555-1234		
(your) Fax#: (301)555-5678		(your) ARIEL IP Address: 111.222.333.444.555

Contact the Access Services Librarian, Document Delivery Services Branch at (301) 504-6503 or via Internet at access@nal.usda.gov with questions or comments about this policy.



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- AD-245 (available from USDA procurement)
- CALS Printout - copy & complete the address & copyright information from the AD245 on to the back of the CALS printout.
- Individual Request Form (IRF)
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Mailing Address:**FAX Number: (301) 504-5675**

National Agricultural Library
Document Delivery Services Branch, Photolab
10301 Baltimore Avenue
Beltsville, MD 20705-2351

E-Mail Request Procedures

E-mail: lending@nal.usda.gov

Electronic mail requests may be sent via the Internet to the address above. Multiple requests may be included in a single message if they are complete unto themselves, contain all of the required data elements and closely resemble the sample below. Each request within a message must be formatted as an individual request complete with name, address, copyright compliance, and request ID number. Use the following standardized number format to identify the requests included in the e-mail message:

1st request sent to NAL on July 25 will be *jul25NAL1*
2nd request sent to NAL on July 25 will be *jul25NAL2*

Each request should cite this identification number on the request form. The number or range of numbers if more than one request is included in the same e-mail message (ex: *jul25NAL1-4*) should also appear in the subject line of the e-mail message. (See back of page for sample e-mail request.)

ARIEL™ Request Procedures

NAL IP Address: ariel.nal.usda.gov

ARIEL™ is a document delivery software package (available from Research Libraries Group) which allows documents to be scanned and sent over the Internet. Requests can be submitted via email, mail, etc., or created on one of the standard forms listed below, scanned and sent to NAL's IP address. If an ARIEL™ package is loaded on to your computer you can receive and print articles at your desktop. If an ARIEL™ address is included in a request, NAL will deliver up to 30 pages per article via ARIEL™. If the article length exceeds 30 pages or cannot be scanned reliably, NAL will deliver the material via Postal Service. For more information about this software (including equipment requirements) visit RLG's ARIEL™ WWW site (http://www.rlg.org/cgi-bin/print_hit_bold.pl/ariel.html).

- over -

The National Agricultural Library accepts requests from other libraries using the OCLC interlibrary loan subsystem. If a library is sending the request by OCLC (Online Computer Library Center) use the code above for NAL.

How to Format Requests

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- Complete mailing information as described below. On e-mail requests this information must be in block format with at least two blank lines above and below so form may be used in a window envelope.
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 - Libraries should include library name and complete mailing address
- Complete citation including verification (source of citation) and NAL call number if available.
- Date after which item is no longer needed.
- Copyright Compliance -- Pre-printed forms must contain your signature to indicate copyright compliance. On e-mail requests include the complete Statement of Copyright Compliance ("I have read the warning on copyright restrictions and accept full responsibility for compliance."), **your full name, date, and telephone number in each request.** Libraries may indicate compliance by including the initials of one statement, either "CCL" for compliance with the copyright law or "CCG" for compliance with Copyright Guidelines or a statement that the request complies with U.S. Copyright Law or other acceptable copyright laws (i.e. IFLA, CLA, etc.). Libraries must also provide authorizing official's name. Requests will be rejected if this information is not included.
- Borrower's Fax number or ARIEL™ IP address (if delivery by either of these methods is desired).

Sample Electronic Mail Request

JUL25NAL1

Date Not Needed After: 8/25/976

Richard Smith
ARS, USDA
Research Station
Heartland, IA 56789

Canadian Journal of Soil Science 1988 v 68(1): 17-27
De Jong, R. Comparison of two soil-water models under semi-arid growing conditions

NAL Call Number: 56.8 C162

Ver: AGRICOLA

I have read the warning on copyright restrictions and accept full responsibility for compliance.

Richard Smith
(your) Fax#: (301)555-5678

7/25/96

Phone# (301)555-1234

(your) ARIEL IP Address: 111.222.333.444.555
